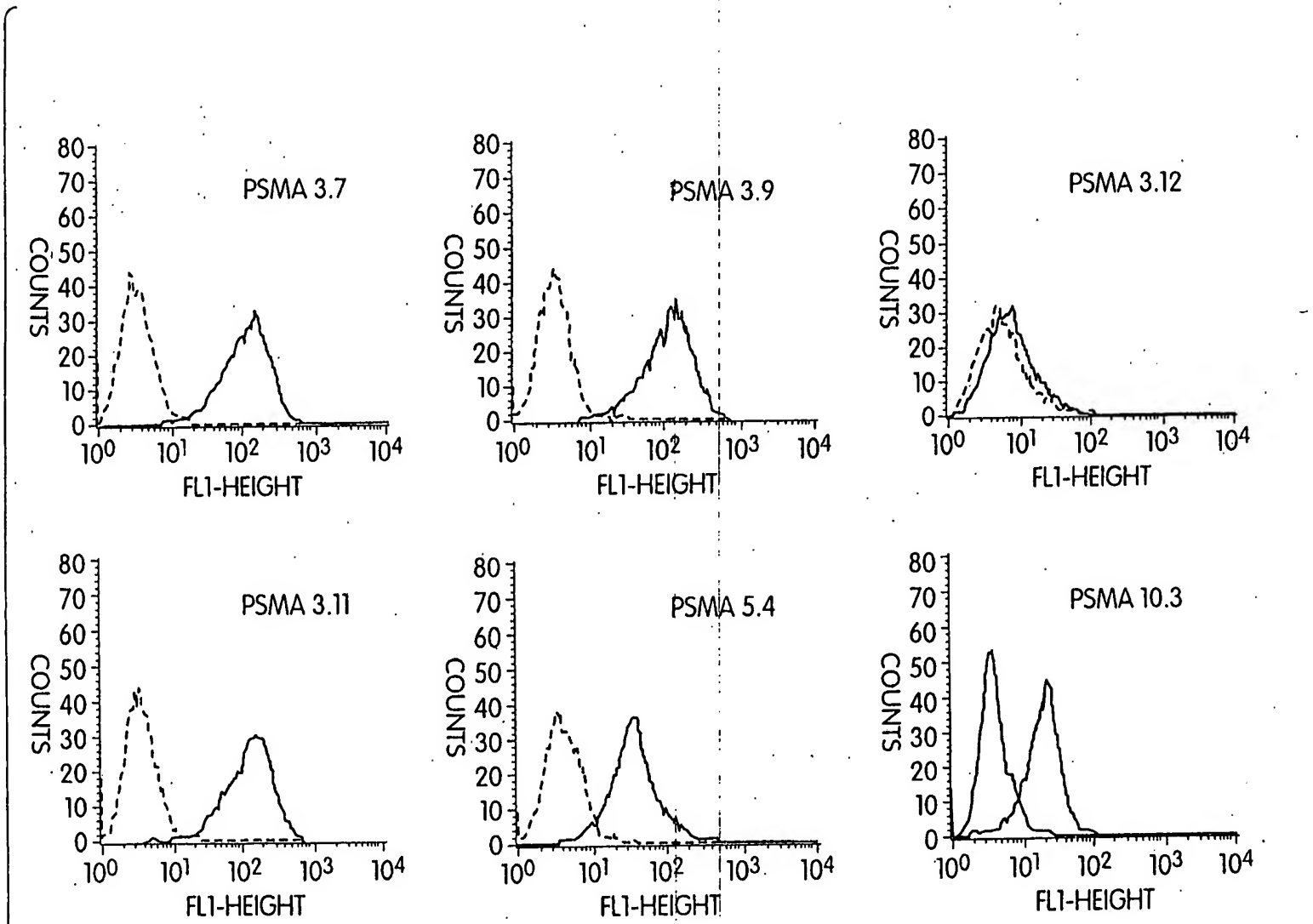


Fig. 1

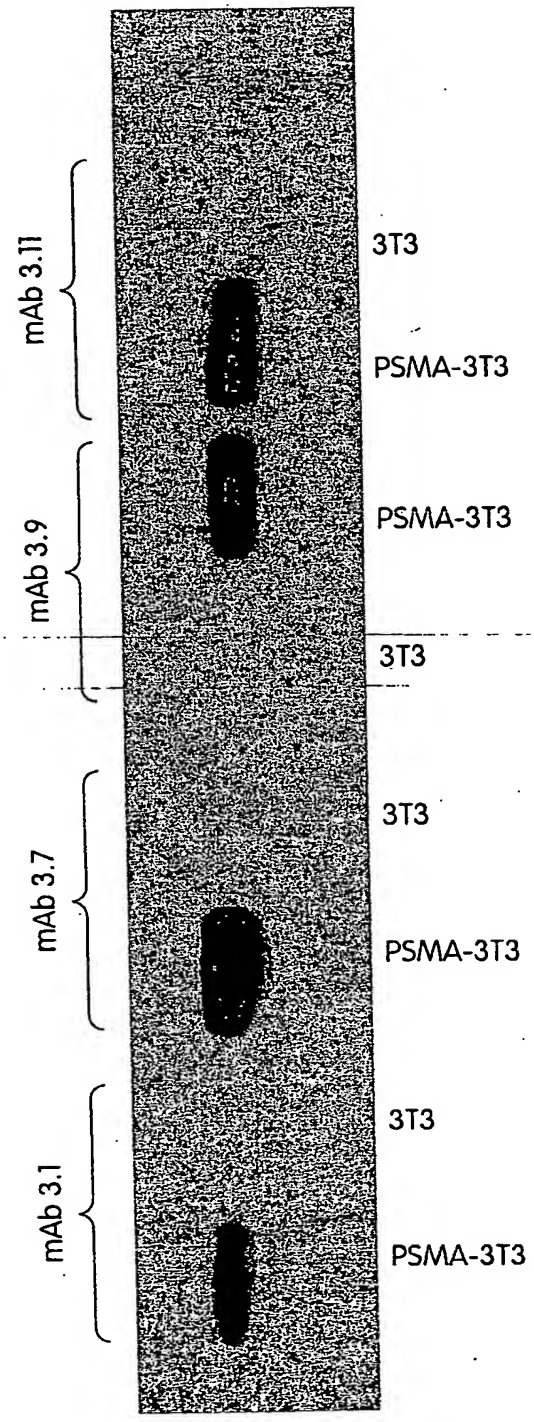

mAb 3.11
3T3
PSMA-3T3
mAb 3.9
PSMA-3T3
3T3
mAb 3.7
3T3
PSMA-3T3
mAb 3.1
3T3
PSMA-3T3

Fig. 2

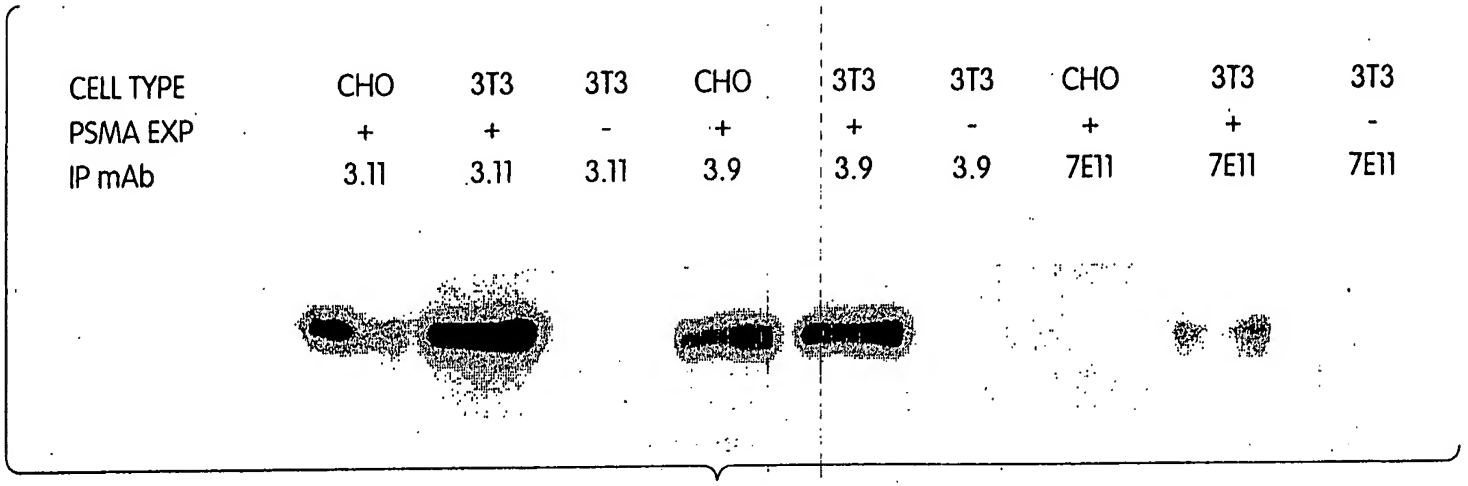

CELL TYPE CHO 3T3 3T3 CHO 3T3 3T3 CHO 3T3 3T3
PSMA EXP + + - + + - + + -
IP mAb 3.11 3.11 3.11 3.9 3.9 3.9 7E11 7E11 7E11

Fig. 3

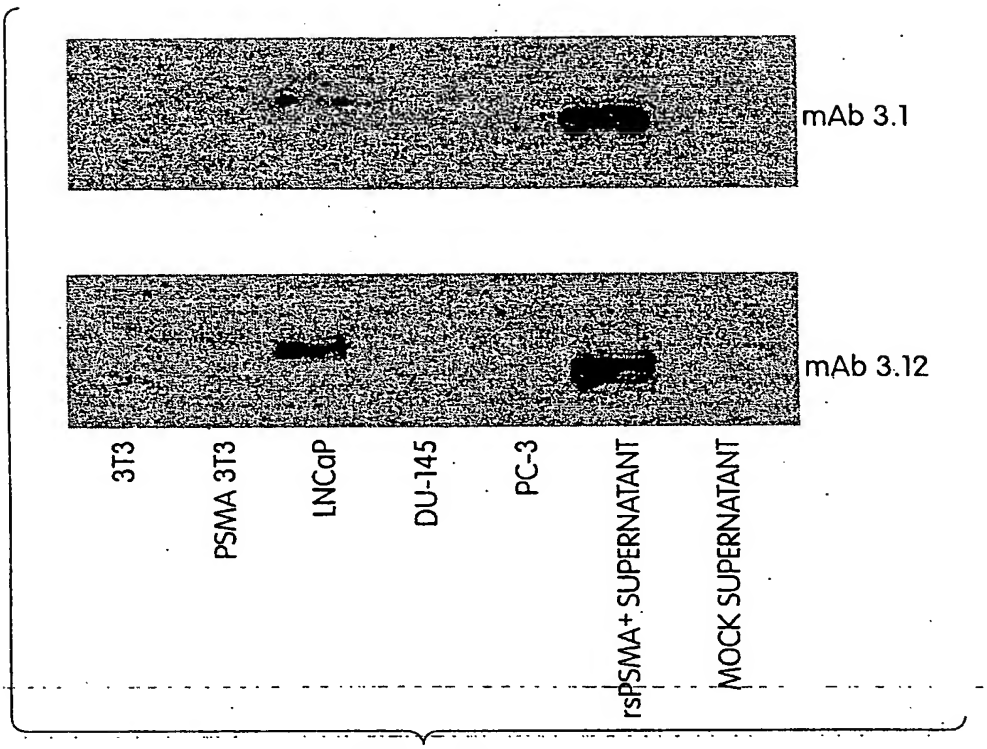

mAb 3.1
mAb 3.12
3T3
PSMA 3T3
LNCaP
DU-145
PC-3
rsPSMA+ SUPERNATANT
MOCK SUPERNATANT

Fig. 4

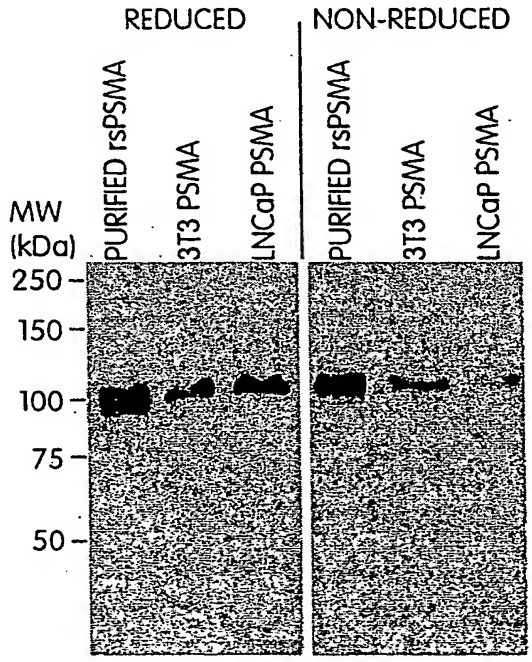

REDUCED
NON-REDUCED
PURIFIED rsPSMA
3T3 PSMA
LNCaP PSMA
PURIFIED rsPSMA
3T3 PSMA
LNCaP PSMA
MW
(kDa)
250
150
100
75
50

Fig. 5

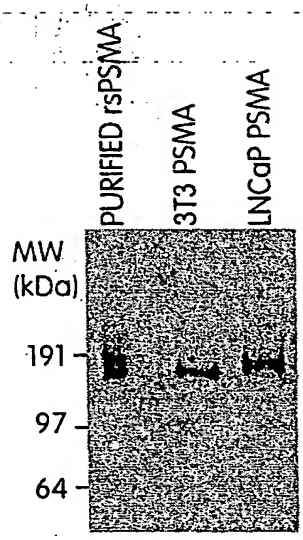


Fig. 6

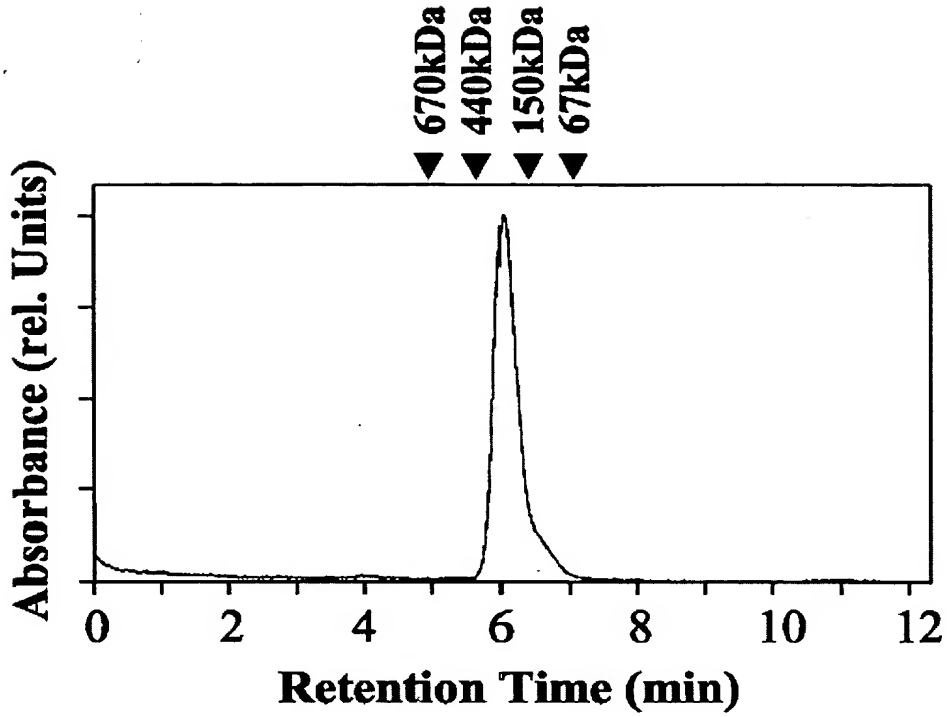

670kDa
440kDa
150kDa
67kDa
Absorbance (rel. Units)
0
2
4
6
8
10
12
Retention Time (min)

Fig. 6B

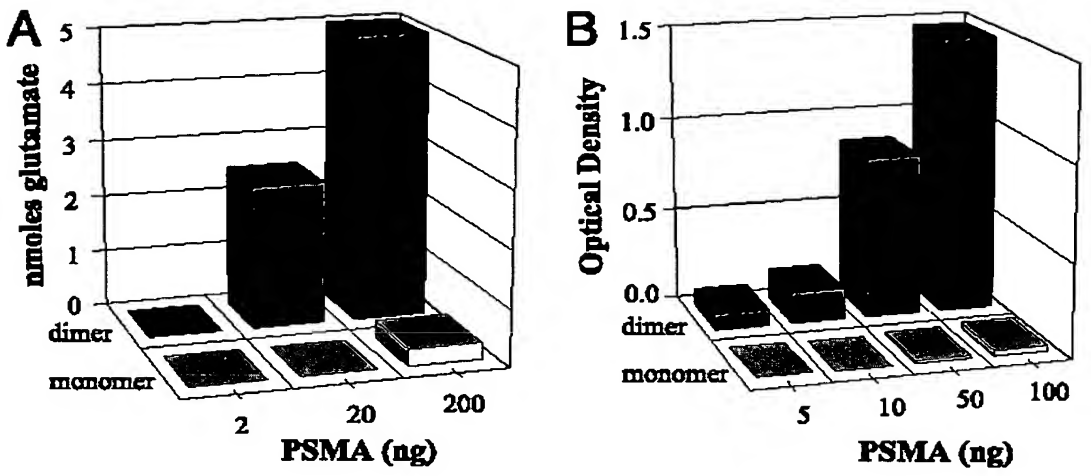

A
nmoles glutamate
5
4
3
2
1
0
dimer
monomer
2
20
200
PSMA (ng)
B
Optical Density
1.5
1.0
0.5
0.0
dimer
monomer
5
10
50
100
PSMA (ng)

Fig. 7

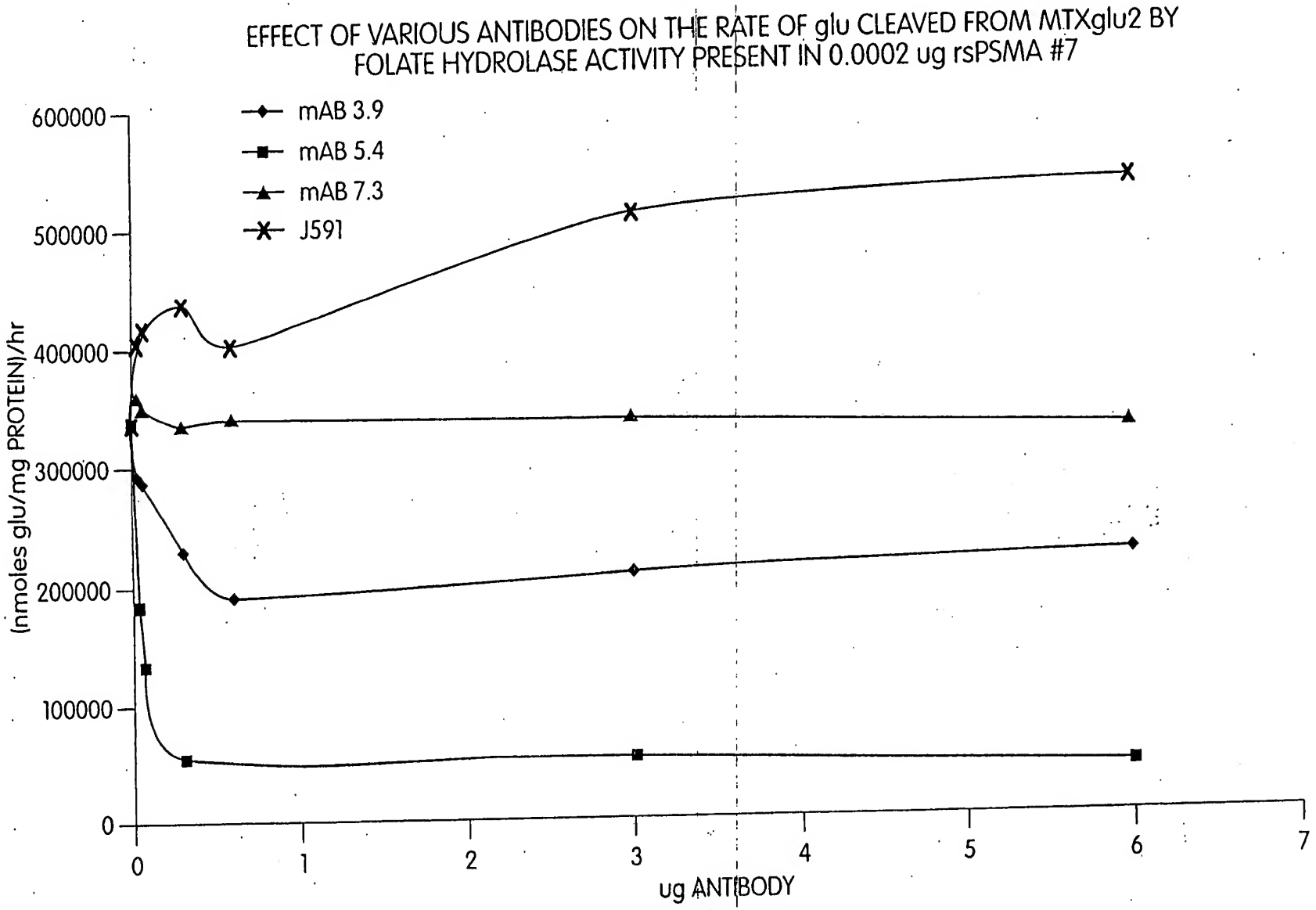

EFFECT OF VARIOUS ANTIBODIES ON THE RATE OF glu CLEAVED FROM MTXglu2 BY FOLATE HYDROLASE ACTIVITY PRESENT IN 0.0002 ug rsPSMA #7
mAB 3.9
mAB 5.4
mAB 7.3
J591
(nmoles glu/mg PROTEIN)/hr
600000
500000
400000
300000
200000
100000
0
0
1
2
3
4
5
6
7
ug ANTIBODY

Fig. 8

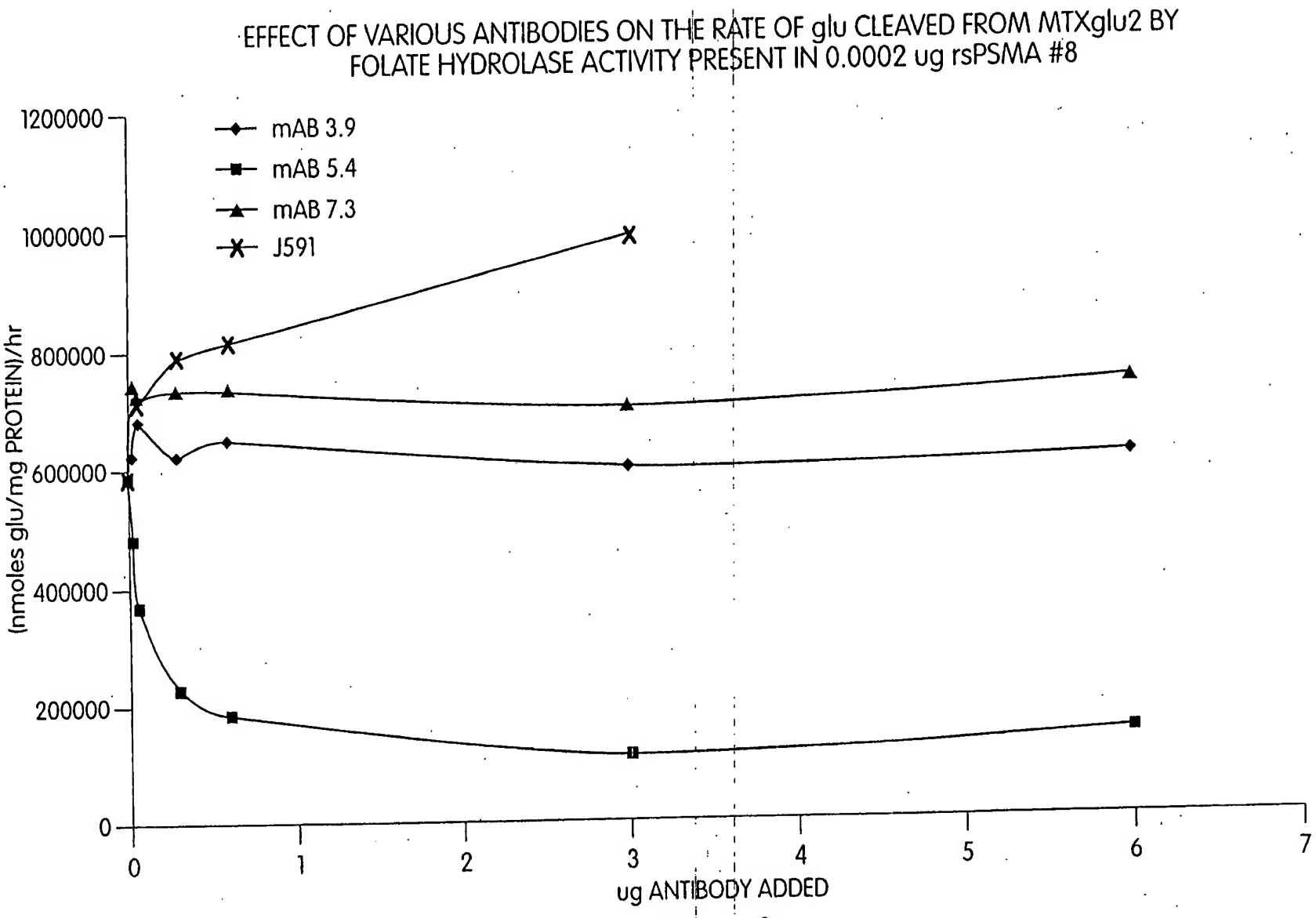

EFFECT OF VARIOUS ANTIBODIES ON THE RATE OF glu CLEAVED FROM MTXglu2 BY FOLATE HYDROLASE ACTIVITY PRESENT IN 0.0002 ug rsPSMA #8
mAB 3.9
mAB 5.4
mAB 7.3
J591
(nmoles glu/mg PROTEIN)/hr
1200000
1000000
800000
600000
400000
200000
0
0
1
2
3
4
5
6
7
ug ANTIBODY ADDED

Fig. 9

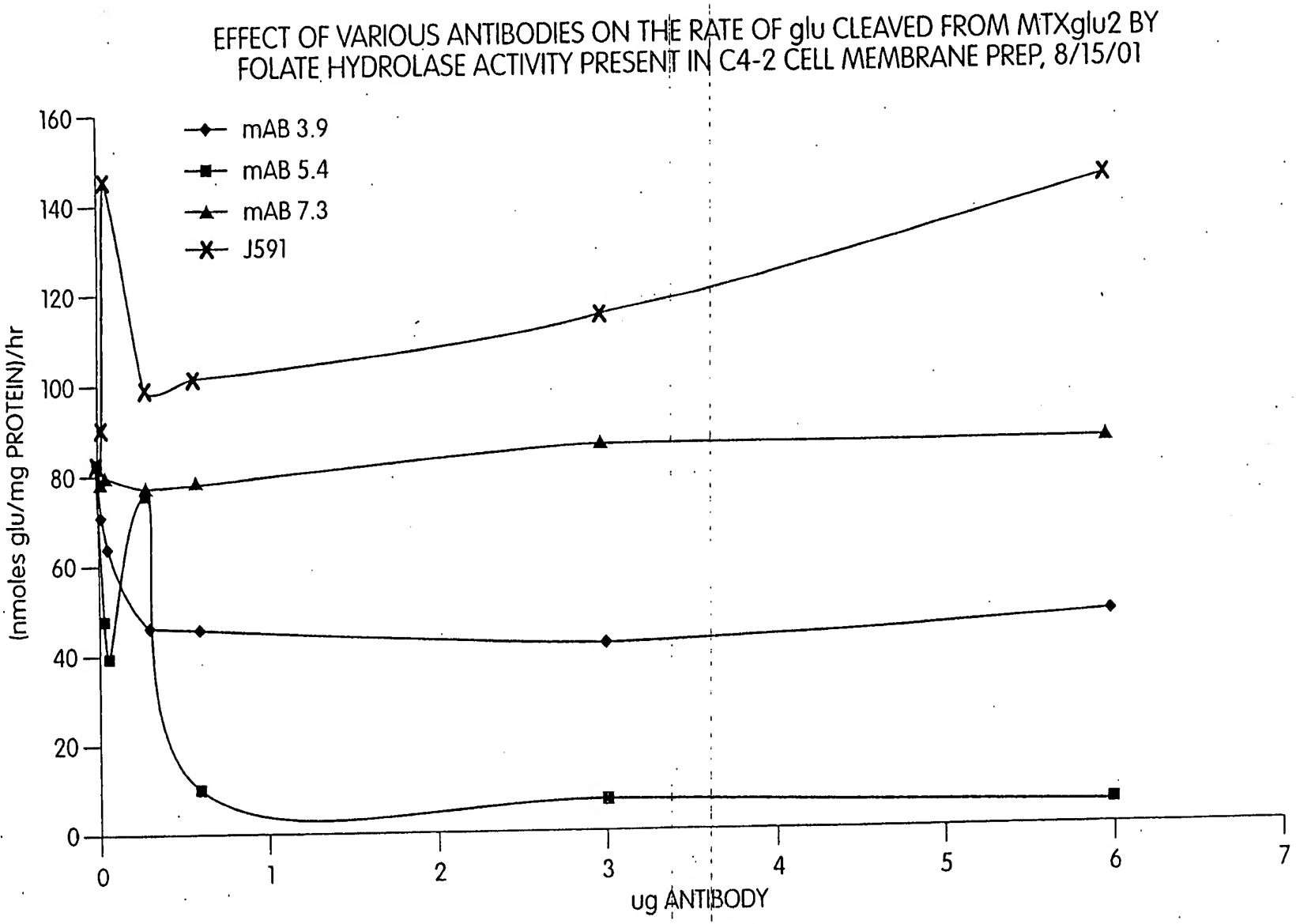
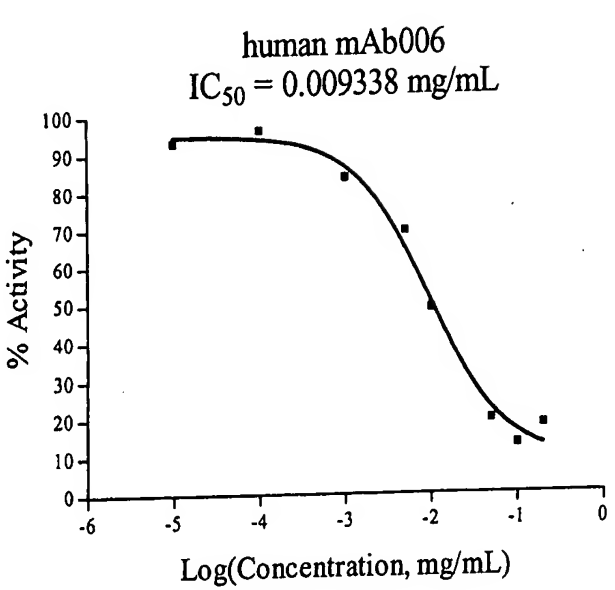
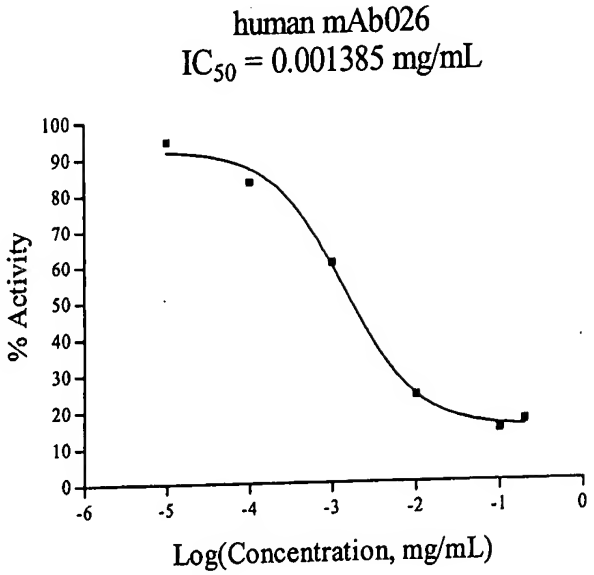
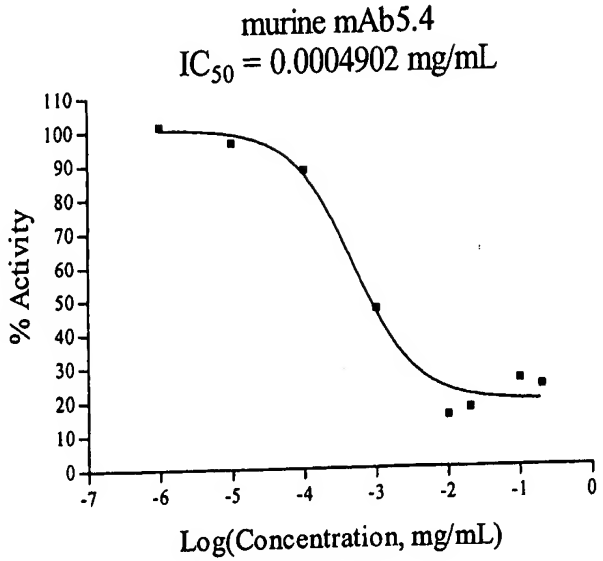

EFFECT OF VARIOUS ANTIBODIES ON THE RATE OF glu CLEAVED FROM MTXglu2 BY FOLATE HYDROLASE ACTIVITY PRESENT IN C4-2 CELL MEMBRANE PREP, 8/15/01
mAB 3.9
mAB 5.4
mAB 7.3
J591
(nmoles glu/mg PROTEIN)/hr
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
ug ANTIBODY

Fig. 10


human mAb006
IC50 = 0.009338 mg/mL
% Activity
Log(Concentration, mg/mL)


human mAb026
IC50 = 0.001385 mg/mL
% Activity
Log(Concentration, mg/mL)


murine mAb5.4
IC50 = 0.0004902 mg/mL
% Activity
Log(Concentration, mg/mL)

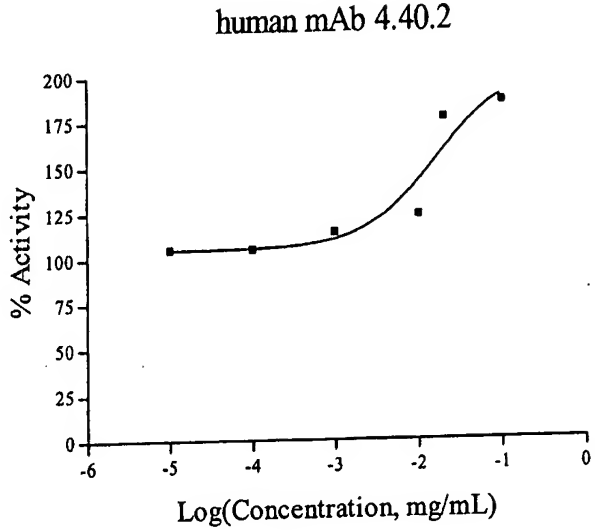
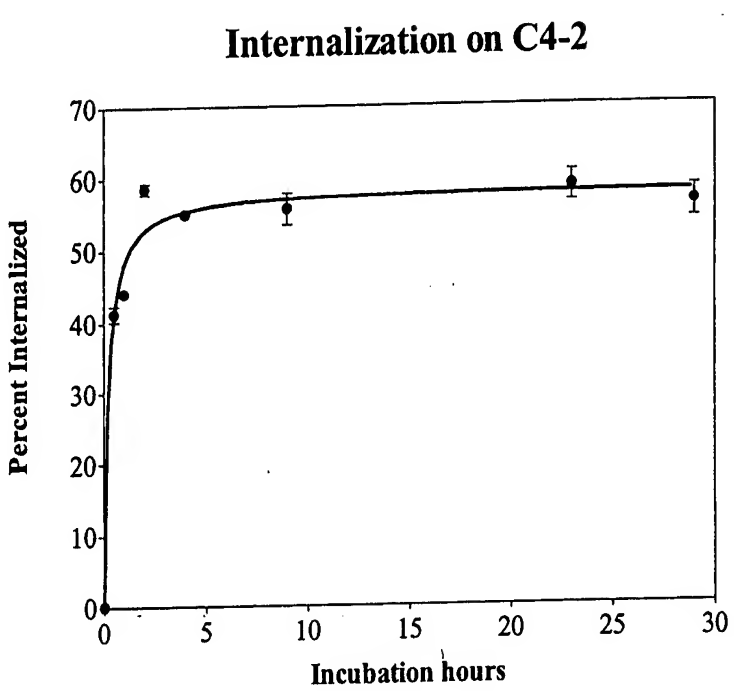

human mAb 4.40.2
% Activity
Log(Concentration, mg/mL)

Fig. 11


Internalization on C4-2
Percent Internalized
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Incubation hours

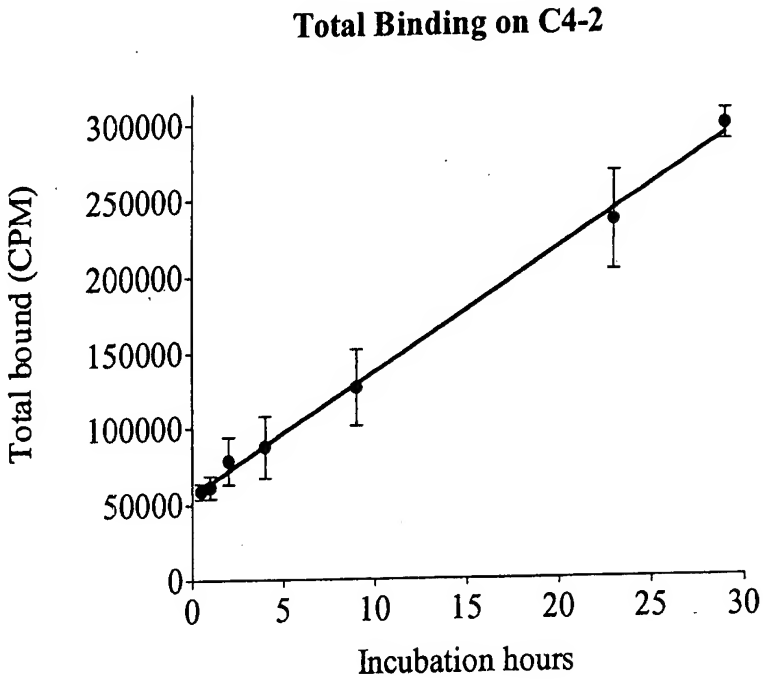

Total Binding on C4-2
Total bound (CPM)
300000
250000
200000
150000
100000
50000
0
0
5
10
15
20
25
30
Incubation hours

Fig. 12

Human IgG1 cl ning – into pcDNA

Construction of pcDNA-huCκ and pcDNA-huIgG1

		PCR product	Vector
Cκ	Sense	5' XbaI HindIII BamHI NheI 3'	5' NheI NotI 3'
	Anti-sense	EcoRI NotI	(pcDNA.neo)
Cγ1	Sense	5' XbaI KpnI HindIII BamHI NheI 3'	5' NheI/PmeI 3'
	Anti-sense	EcoRI XhoI PmeI	(pcDNA Hygro)

Construction of pcDNA-Ab (V-C cassette)

		PCR product	Vector
Vκ	Sense	BglII or BamHI (if necessary)*	5' BamHI NheI 3'
	Anti-sense	NheI	(pcDNA-huCκ)
Vγ1	Sense	BglII or BamHI (if necessary)*	5' BamHI NheI 3'
	Anti-sense	XbaI	(pcDNA-huIgG1)

* BamHI primer is used if the V region has an internal BglII site

Human IgG cloning – V-C cassette from pcDNA into "production" vector

	Insert from pcDNA
Igκ	5' HindIII or BamHI (if alternate sense primer used)
	3' EcoRI, NotI, XhoI, XbaI or PmeI
IgG1	5' KpnI, HindIII or BamHI (if alternate sense primer used)
	3' EcoRI*, XhoI or PmeI

* 2nd EcoRI site present in hygromycin resistance gene

Primers used for V region amplification

Vκ-sense:

5' GA**AGATCT**CACC ATG + 20-23 bp leader sequence 3'
BglII Kozak

Vκ anti-sense (reverse/complementary):

5' AACTA **GCT AGC** AGT TCC AGA TTT CAA CTG CTC ATC AGA T 3'
S A T G S K L Q E D S (aa. 23-13 Cκ)
NheI

Cloning site of NheI codes for A S - therefore no amino acid change due to cloning.

Vγ-sense:

5' GA**AGATCT**CACC ATG + 17-29bp leader sequence 3'
BglII Kozak

Vγ anti-sense(reverse/complementary):

5' GC **TCT AGA** GGG TGC CAG GGG GAA GAC CGA T 3'
(R) S P A L P F V S (aa. 14-7 Cγ1)
XbaI

Cloning into
5' CG **GCT AGC**
S (A)

Cloning site junction of XbaI/NheI (TCT AGC) codes for S S - therefore no amino acid change due to cloning.

Fig. 13

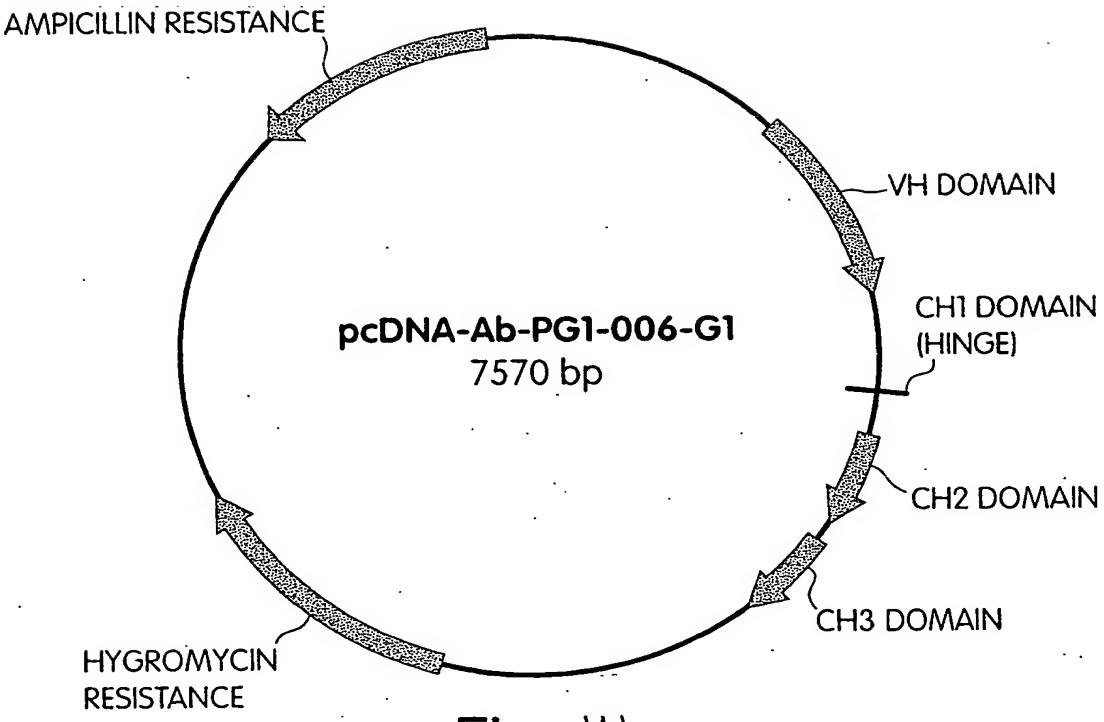

AMPICILLIN RESISTANCE
VH DOMAIN
CH1 DOMAIN
(HINGE)
pcDNA-Ab-PG1-006-G1
7570 bp
CH2 DOMAIN
CH3 DOMAIN
HYGROMYCIN
RESISTANCE

Fig. 14

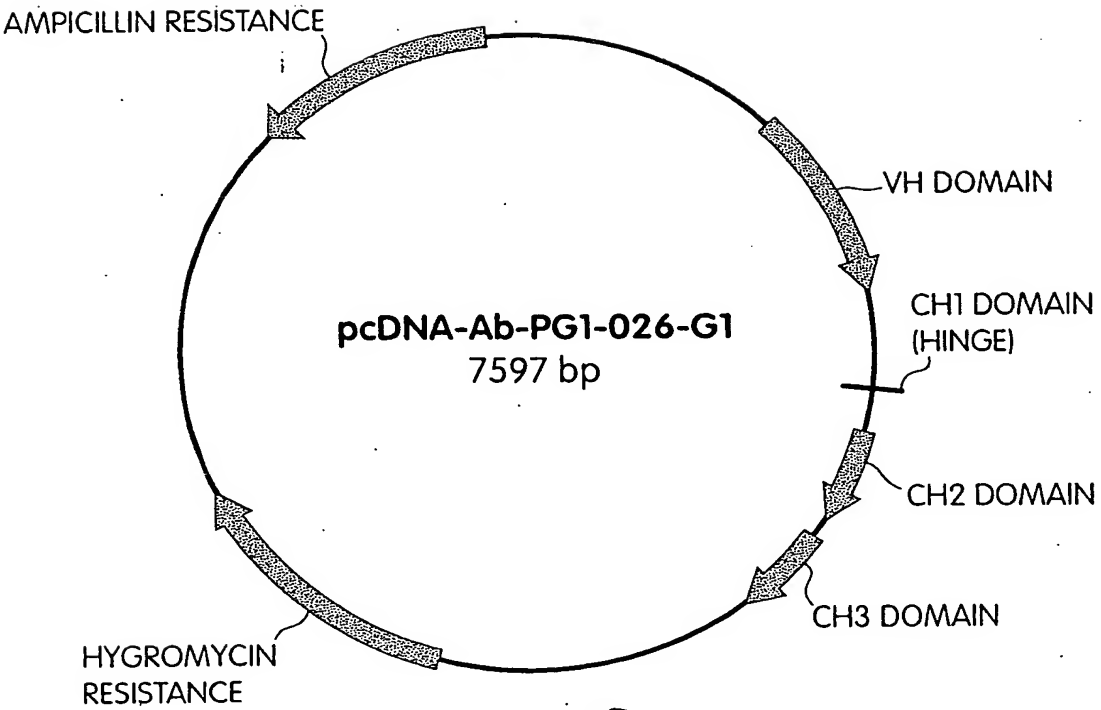

AMPICILLIN RESISTANCE
VH DOMAIN
CH1 DOMAIN
(HINGE)
pcDNA-Ab-PG1-026-G1
7597 bp
CH2 DOMAIN
CH3 DOMAIN
HYGROMYCIN
RESISTANCE

Fig. 15

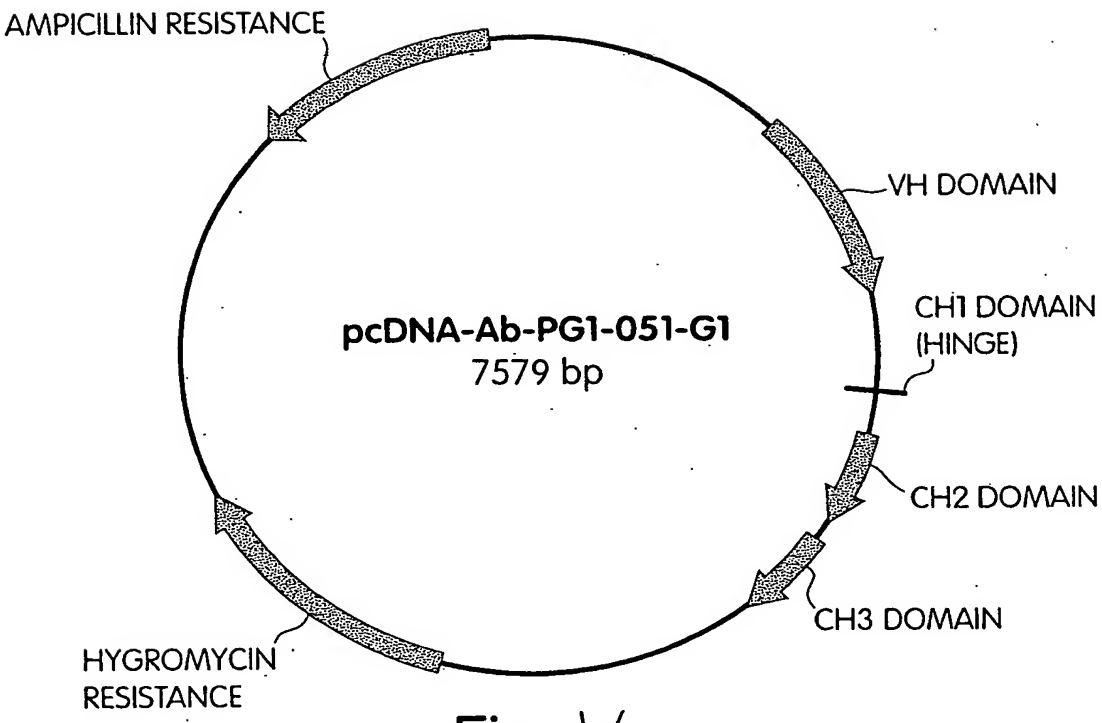

AMPICILLIN RESISTANCE
VH DOMAIN
CH1 DOMAIN
(HINGE)
pcDNA-Ab-PG1-051-G1
7579 bp
CH2 DOMAIN
CH3 DOMAIN
HYGROMYCIN
RESISTANCE

Fig. 16

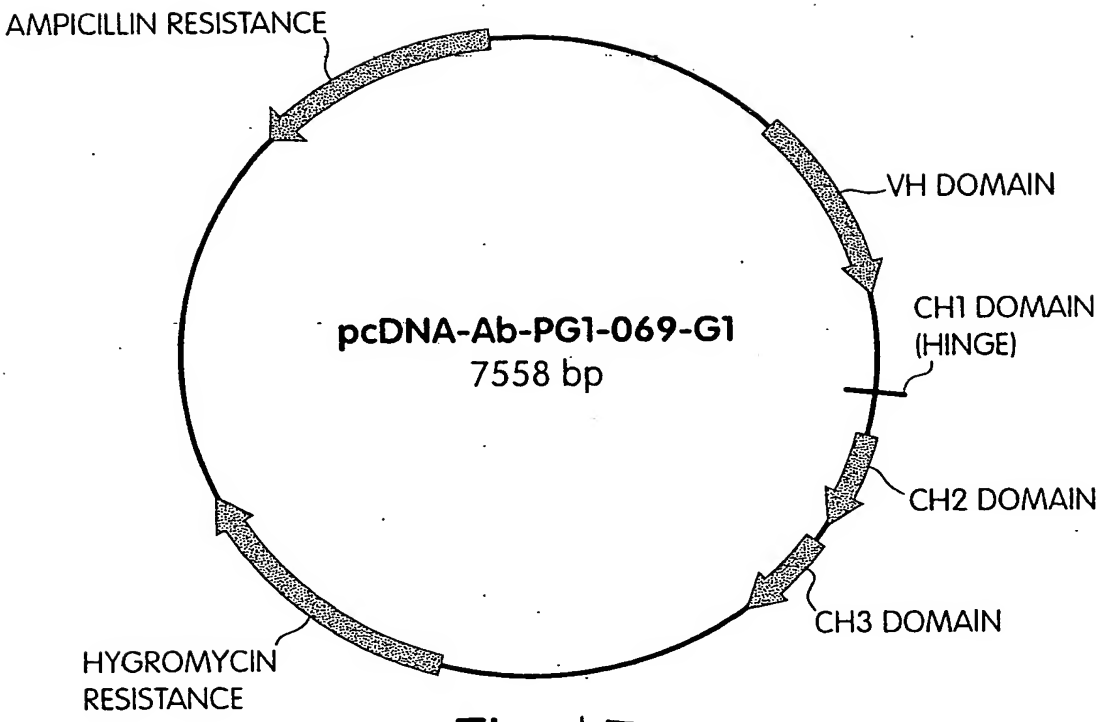

AMPICILLIN RESISTANCE
VH DOMAIN
CH1 DOMAIN
(HINGE)
pcDNA-Ab-PG1-069-G1
7558 bp
CH2 DOMAIN
CH3 DOMAIN
HYGROMYCIN
RESISTANCE

Fig. 17

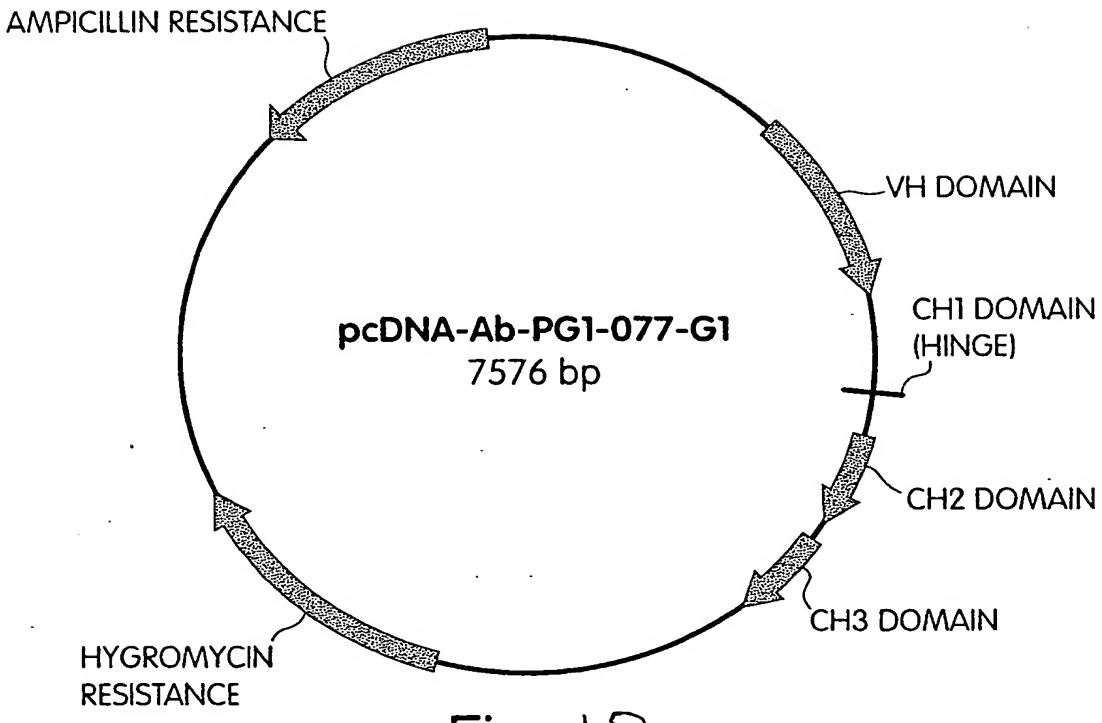

AMPICILLIN RESISTANCE
VH DOMAIN
CH1 DOMAIN
(HINGE)
pcDNA-Ab-PG1-077-G1
7576 bp
CH2 DOMAIN
CH3 DOMAIN
HYGROMYCIN
RESISTANCE

Fig. 18

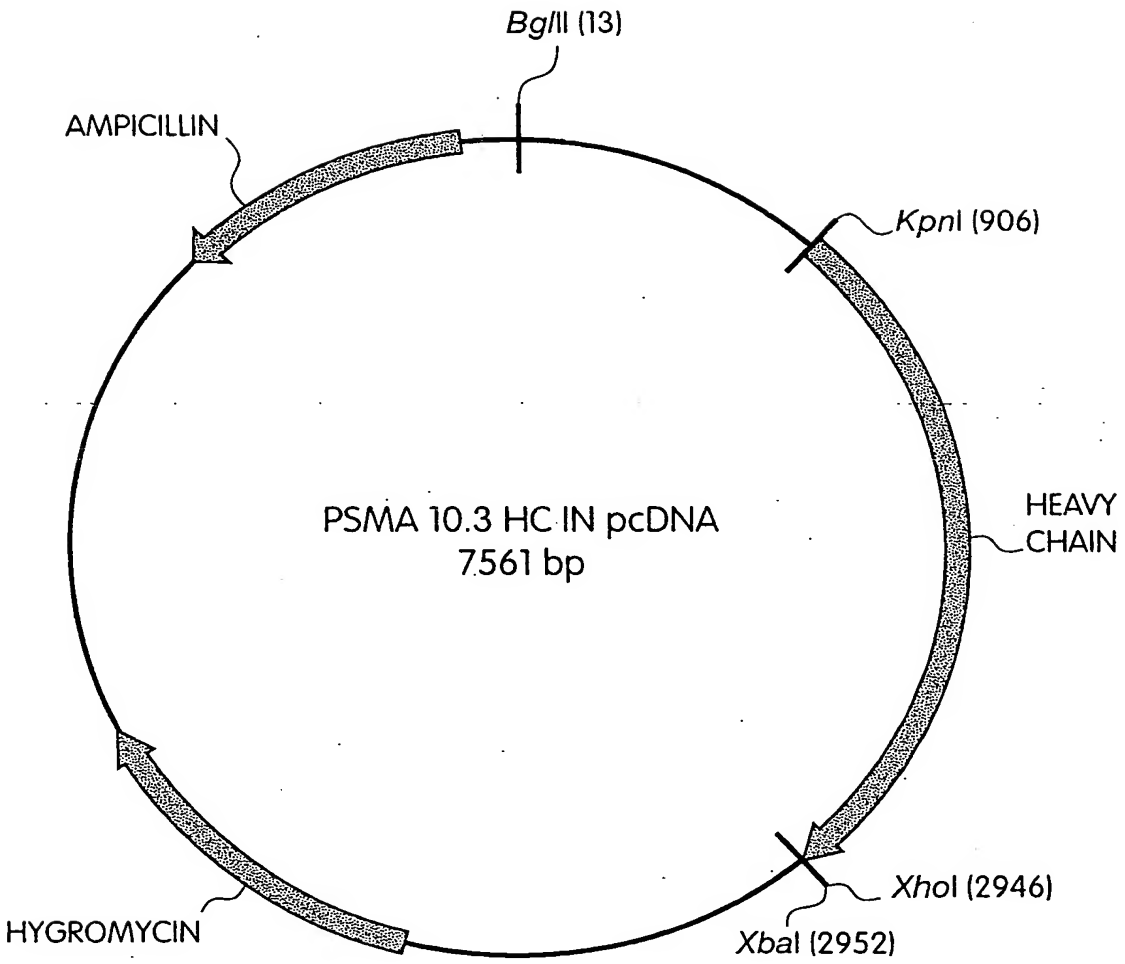

BglII (13)
AMPICILLIN
KpnI (906)
PSMA 10.3 HC IN pcDNA
7561 bp
HEAVY
CHAIN
XhoI (2946)
XbaI (2952)
HYGROMYCIN

Fig. 19

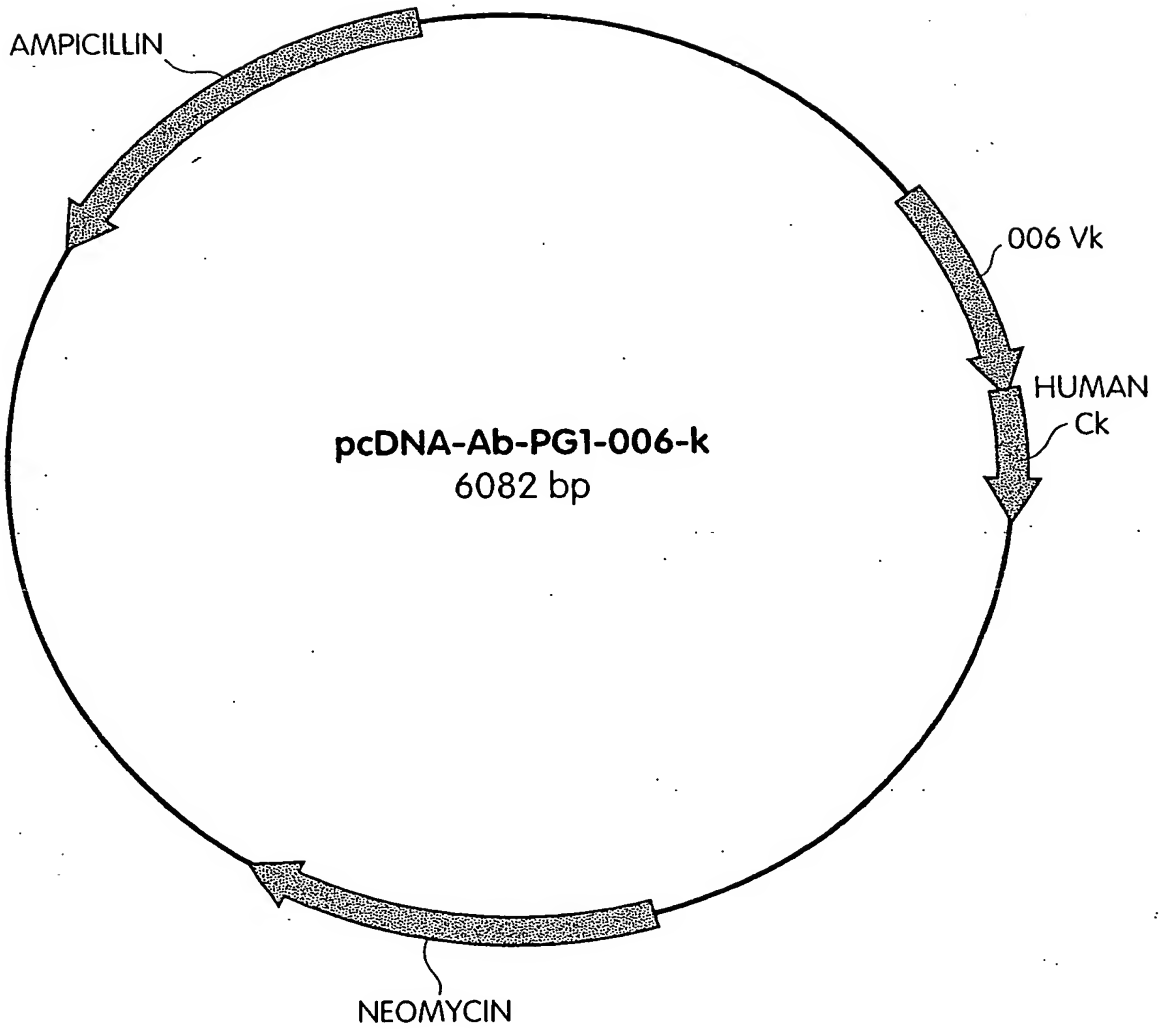

AMPICILLIN
006 Vk
HUMAN
Ck
pcDNA-Ab-PG1-006-k
6082 bp
NEOMYCIN

Fig. 20

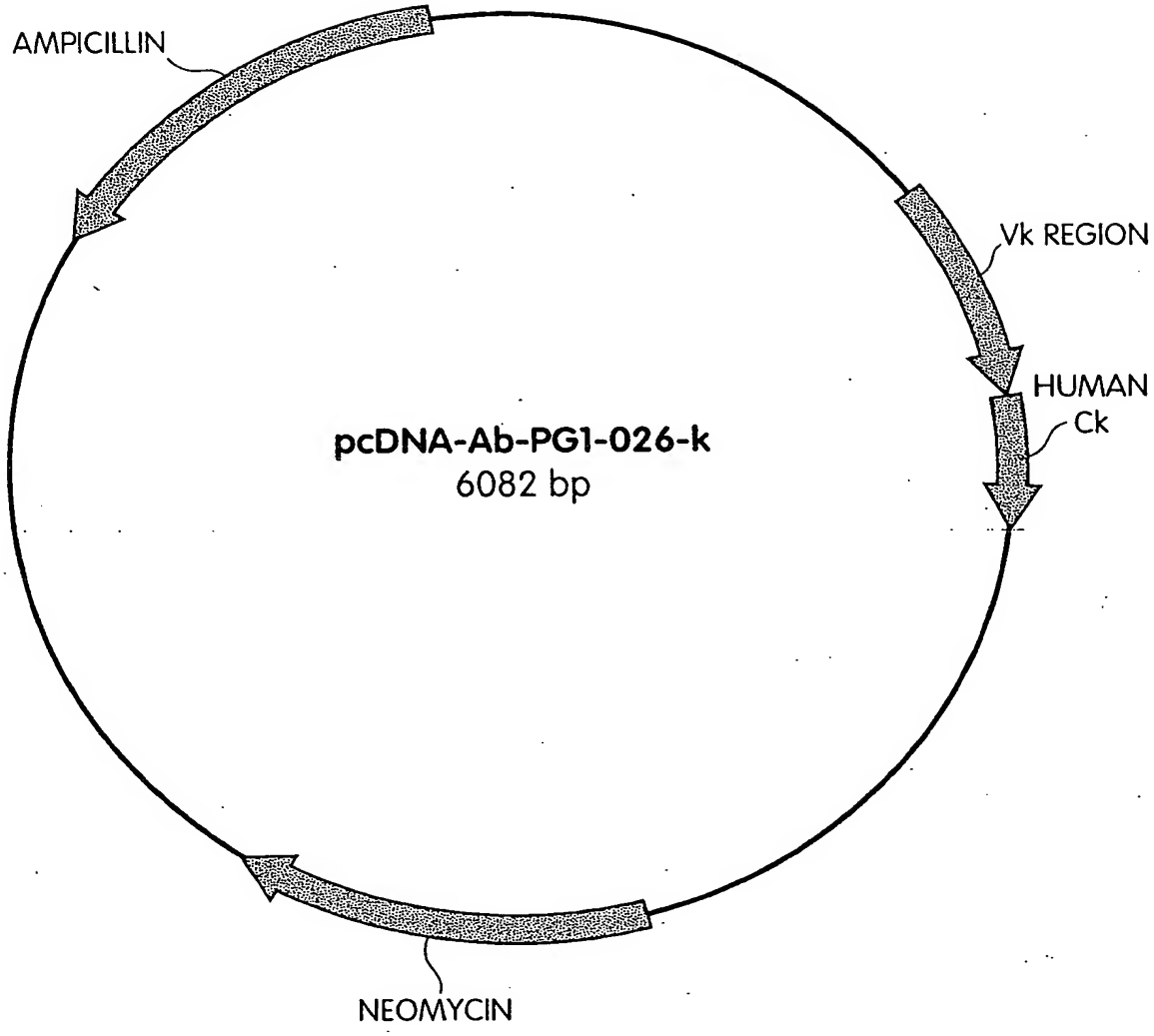

AMPICILLIN
Vk REGION
HUMAN
Ck
pcDNA-Ab-PG1-026-k
6082 bp
NEOMYCIN

Fig. 21

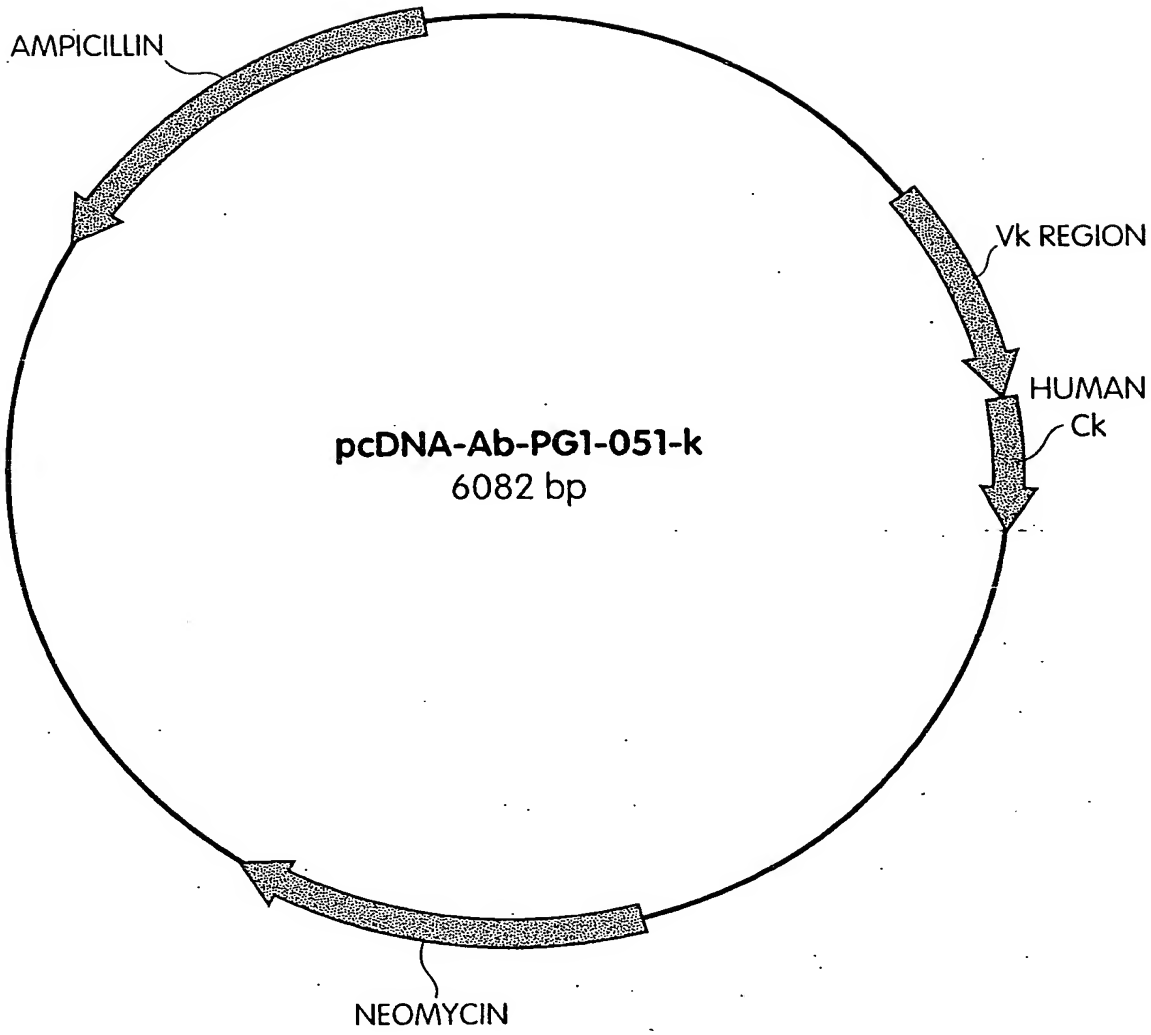

AMPICILLIN
Vk REGION
HUMAN
Ck
pcDNA-Ab-PG1-051-k
6082 bp
NEOMYCIN

Fig. 22

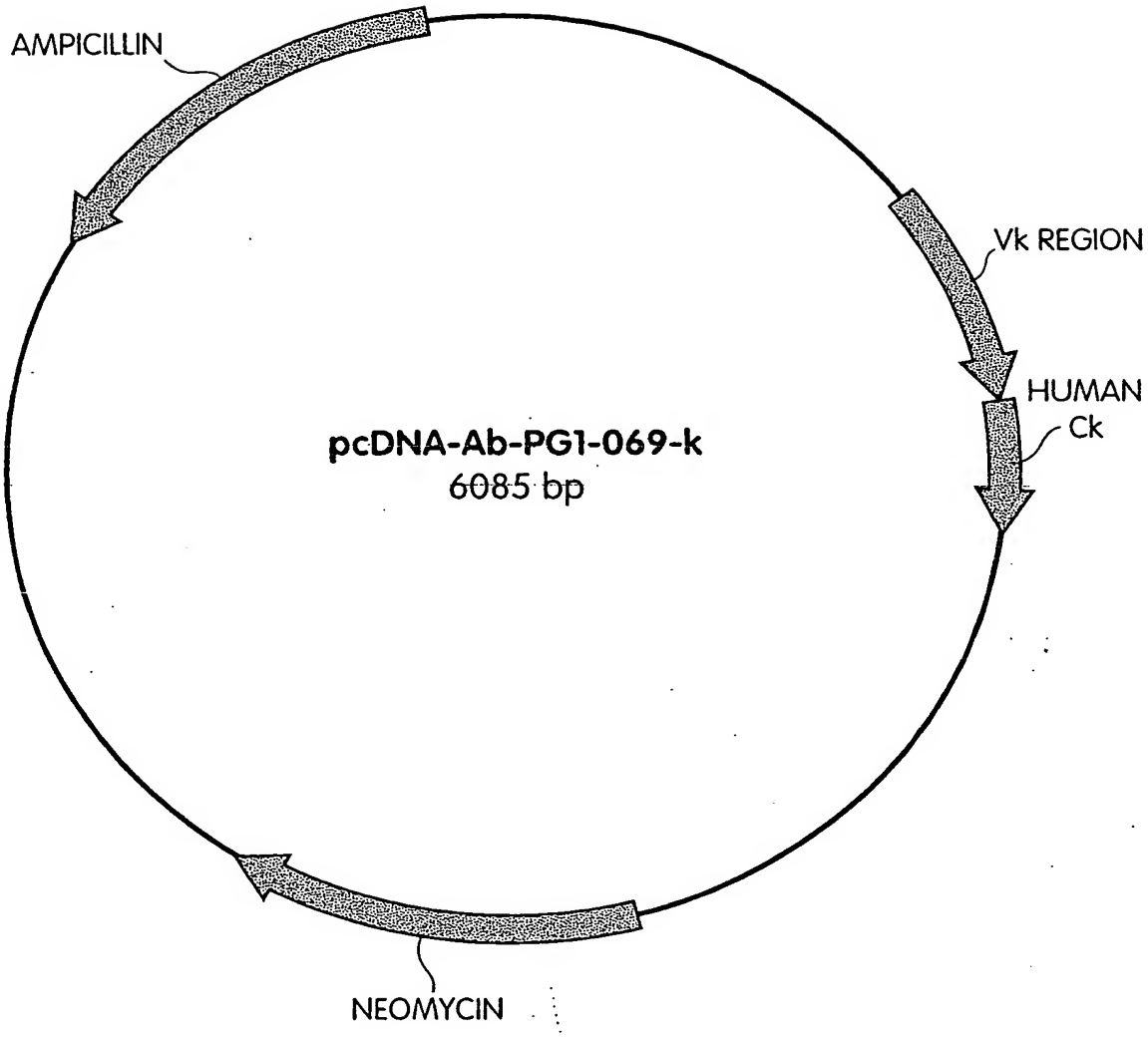

AMPICILLIN
Vk REGION
HUMAN
Ck
pcDNA-Ab-PG1-069-k
6085 bp
NEOMYCIN

Fig. 23

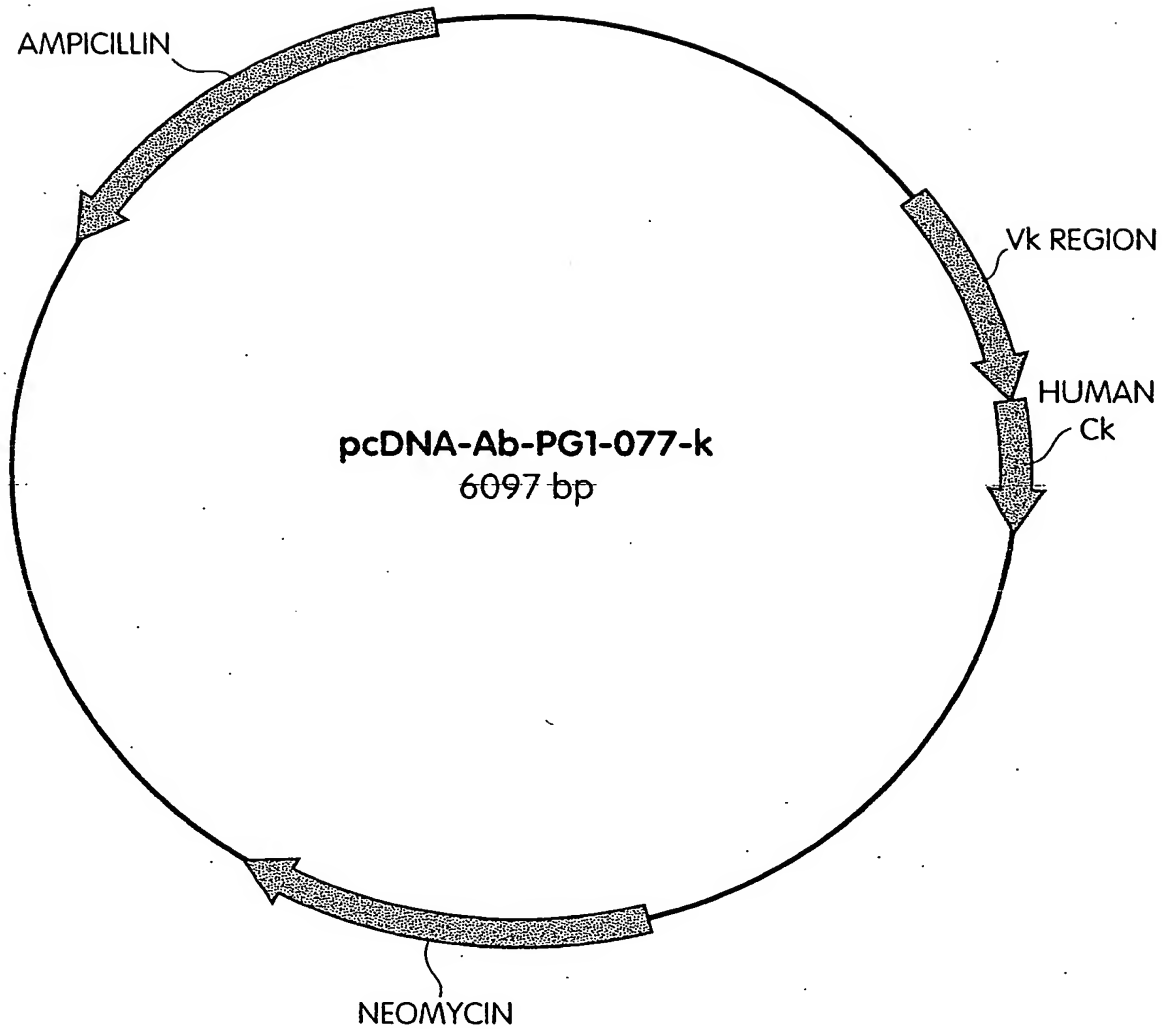

AMPICILLIN
Vk REGION
HUMAN
Ck
pcDNA-Ab-PG1-077-k
6097 bp
NEOMYCIN

Fig. 24

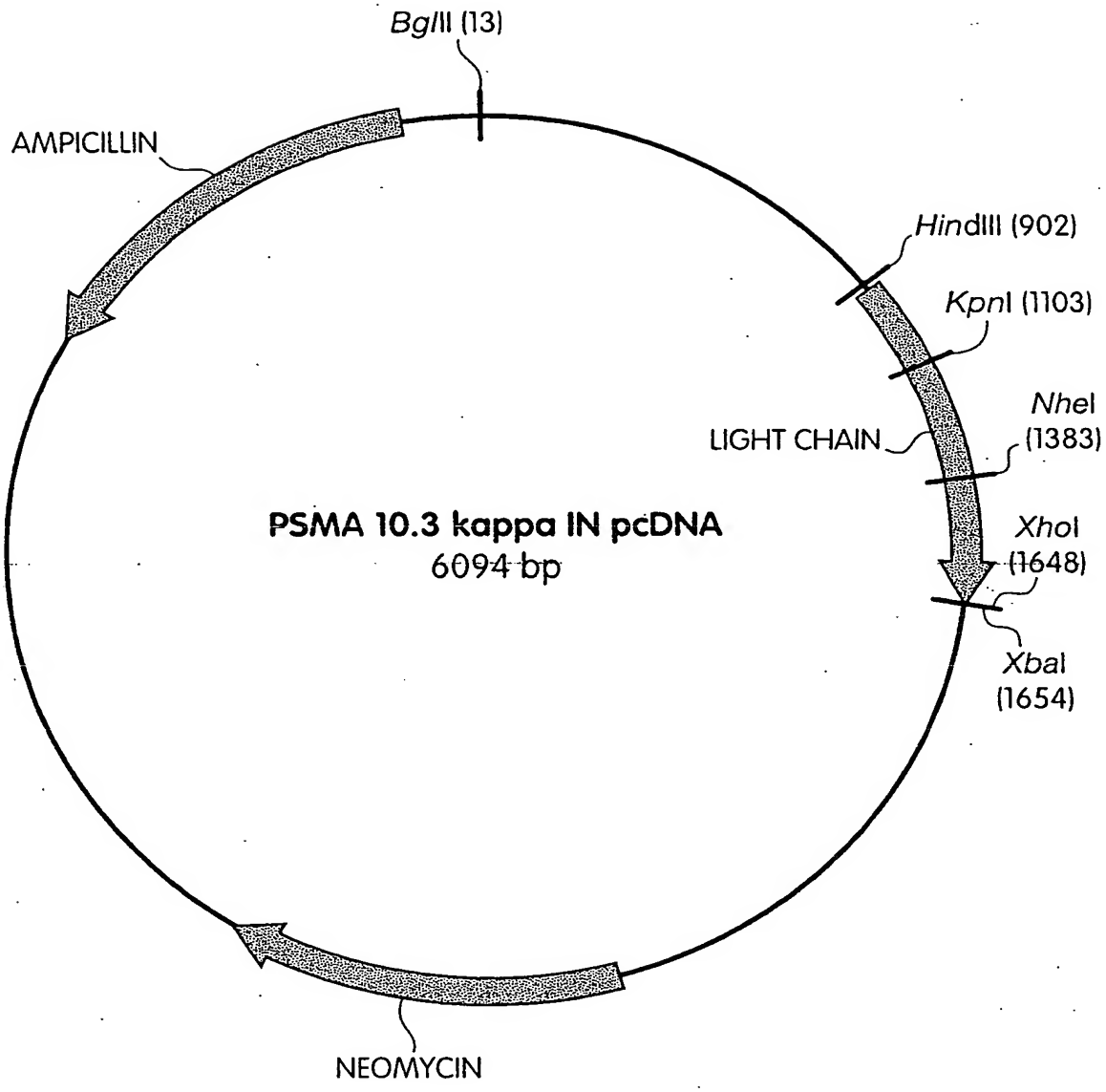

BglII (13)
AMPICILLIN
HindIII (902)
KpnI (1103)
NheI
(1383)
LIGHT CHAIN
PSMA 10.3 kappa IN pcDNA
6094 bp
XhoI
(1648)
XbaI
(1654)
NEOMYCIN

Fig. 25

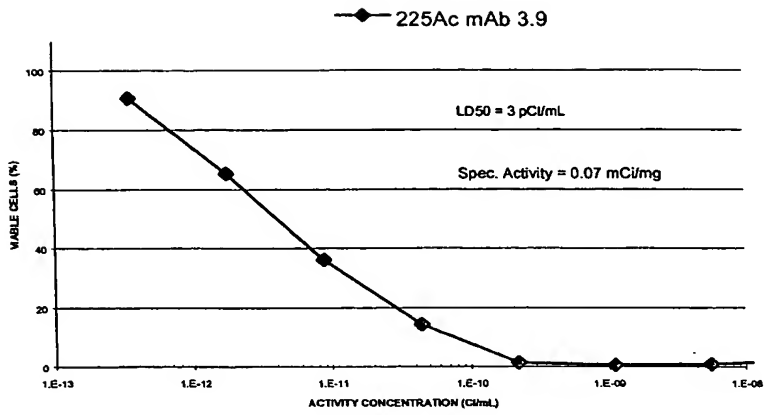

225Ac mAb 3.9
LD50 = 3 pCi/mL
Spec. Activity = 0.07 mCi/mg
VIABLE CELLS (%)
100
80
60
40
20
0
1.E-13
1.E-12
1.E-11
1.E-10
1.E-09
1.E-08
ACTIVITY CONCENTRATION (Ci/mL)

Fig. 2b

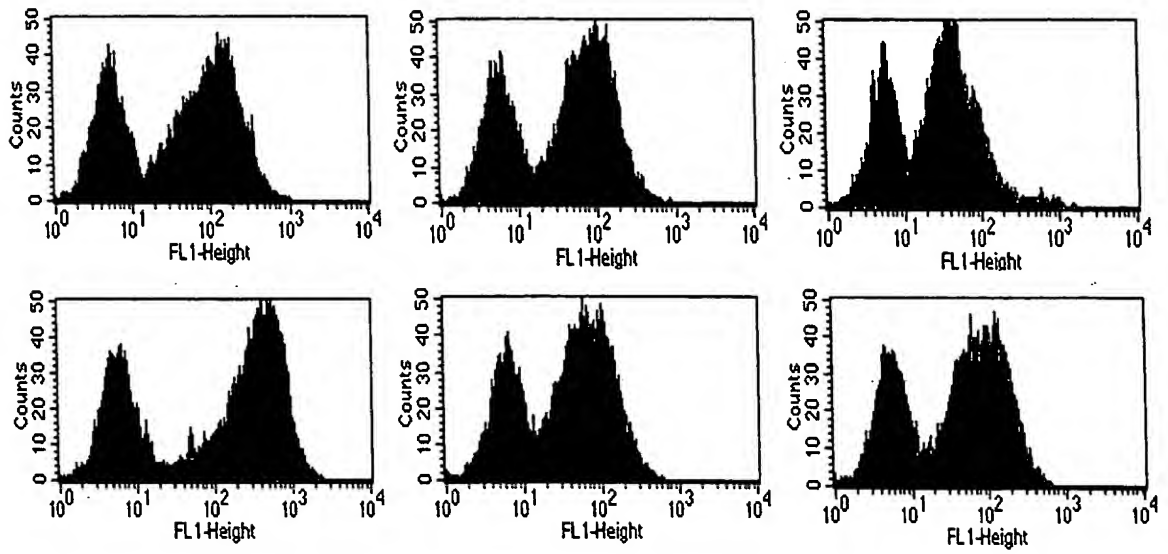


Fig. 27

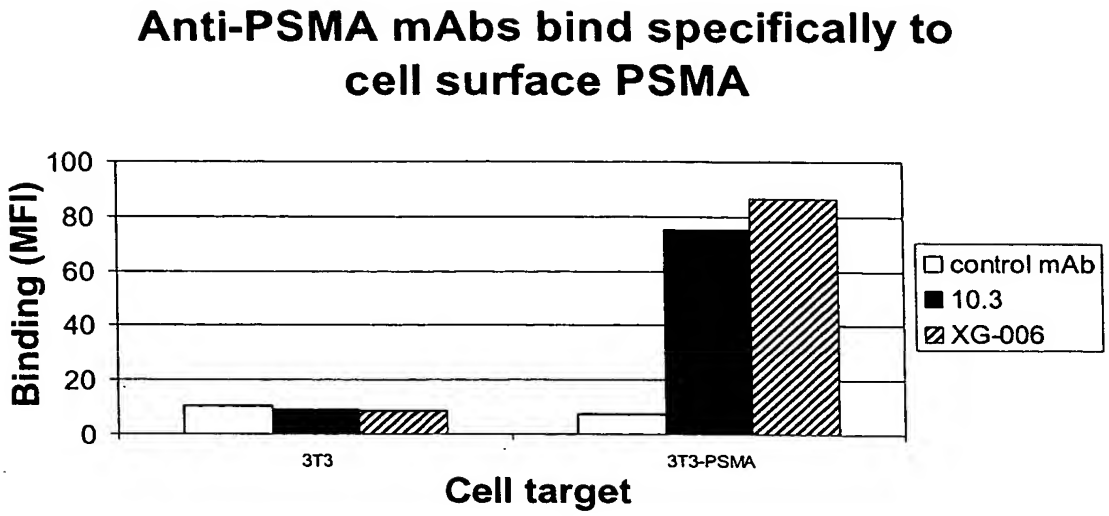

Anti-PSMA mAbs bind specifically to cell surface PSMA
Binding (MFI)
100
80
60
40
20
0
3T3
3T3-PSMA
Cell target
control mAb
10.3
XG-006

Fig. 28A

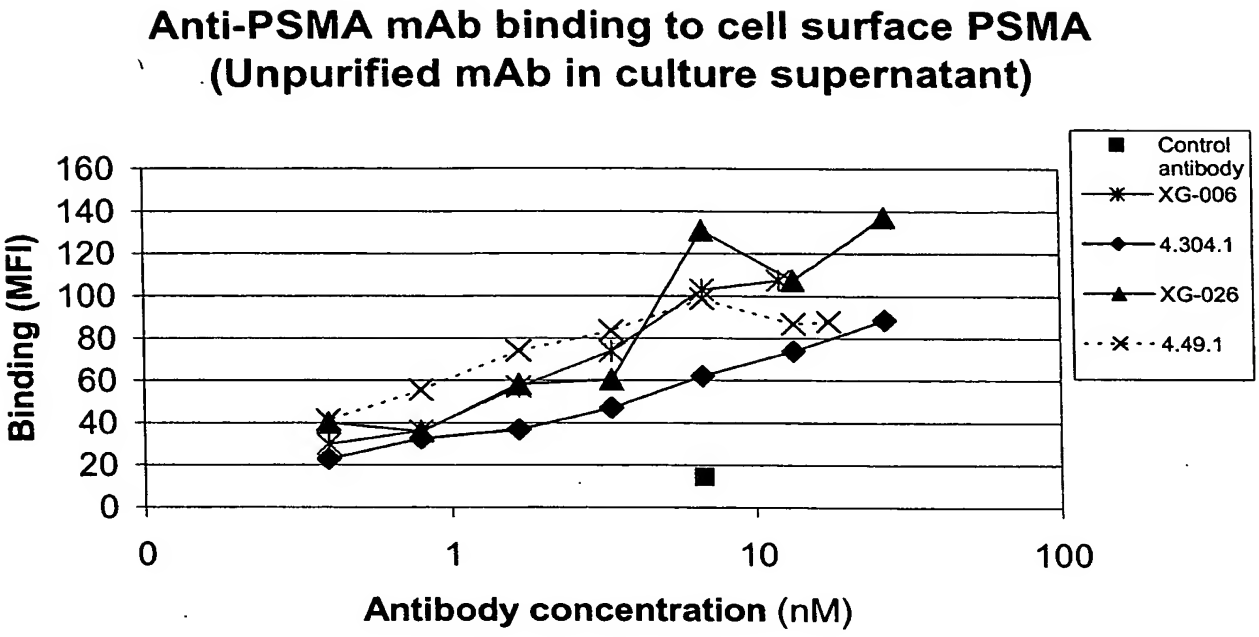

Anti-PSMA mAb binding to cell surface PSMA
(Unpurified mAb in culture supernatant)
Binding (MFI)
160
140
120
100
80
60
40
20
0
0
1
10
100
Antibody concentration (nM)
Control antibody
XG-006
4.304.1
XG-026
4.49.1

Fig. 28B

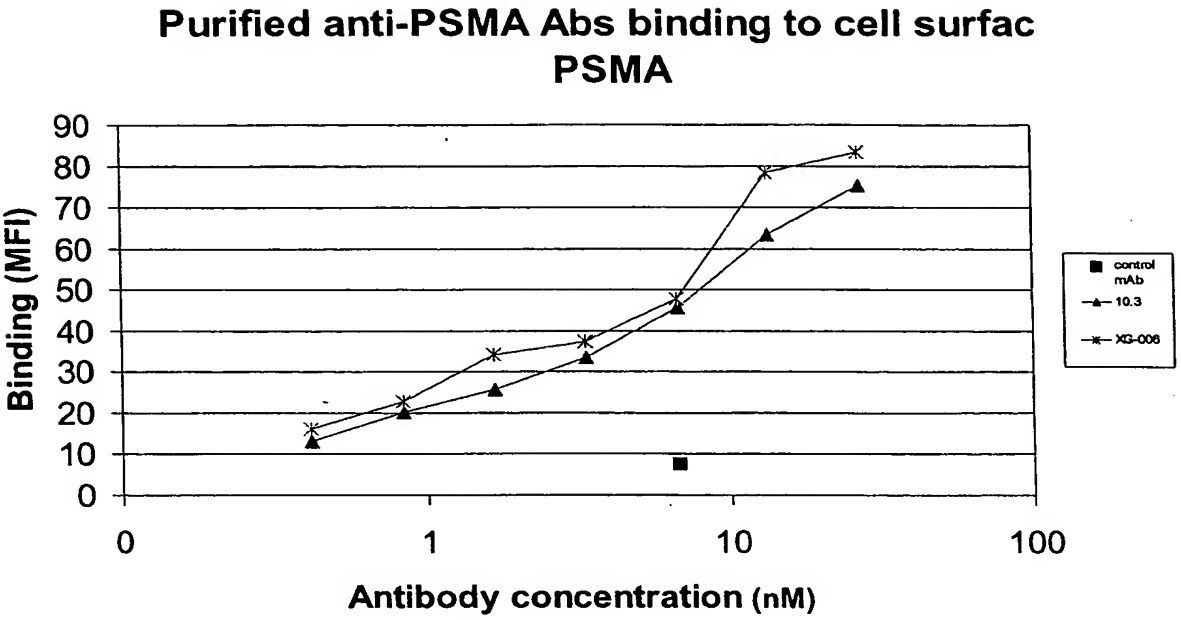
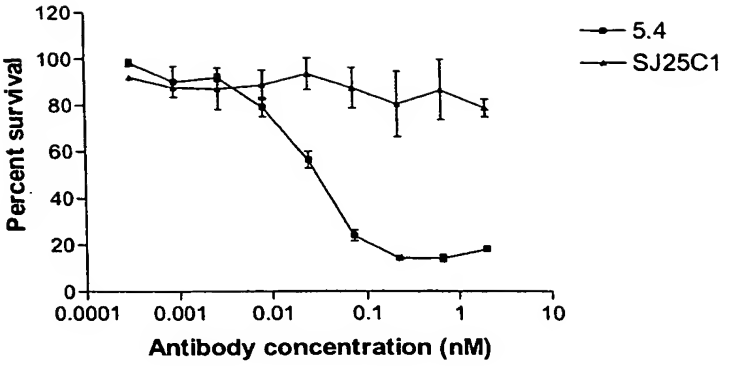
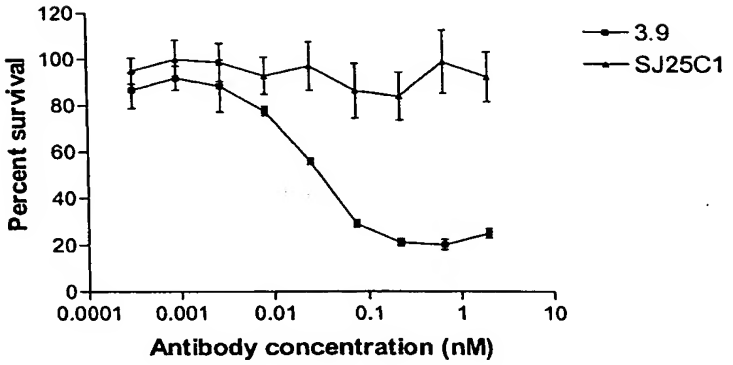

Purified anti-PSMA Abs binding to cell surfac PSMA
Binding (MFI)
90
80
70
60
50
40
30
20
10
0
0
1
10
100
Antibody concentration (nM)
control mAb
10.3
XG-006

Fig. 28C


120
100
80
60
40
20
0
Percent survival
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
5.4
SJ25C1


120
100
80
60
40
20
0
Percent survival
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
3.9
SJ25C1

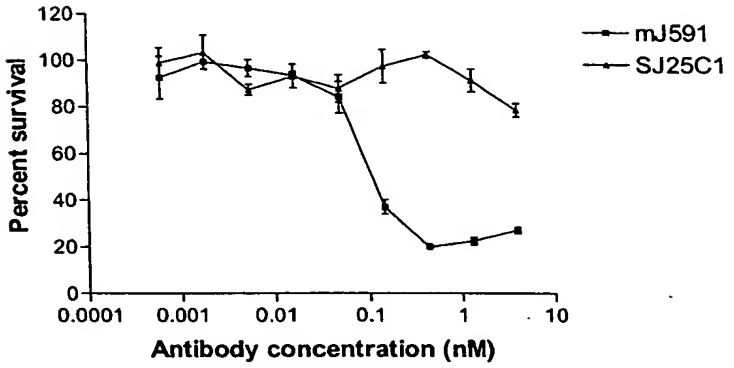
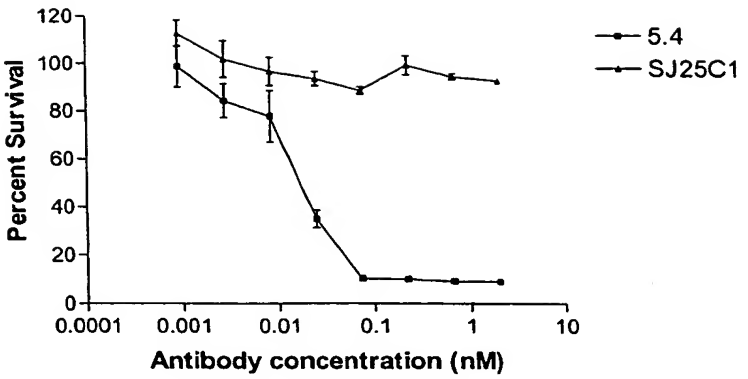
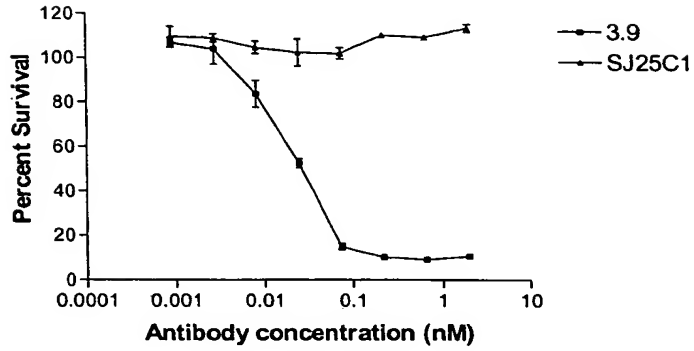

120
100
80
60
40
20
0
Percent survival
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
mJ591
SJ25C1

Fig. 29


Percent Survival
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
5.4
SJ25C1


Percent Survival
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
3.9
SJ25C1

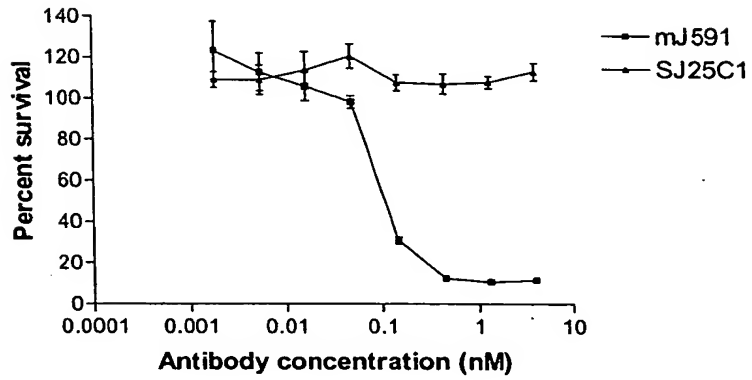

Percent survival
140
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
mJ591
SJ25C1

Fig. 30

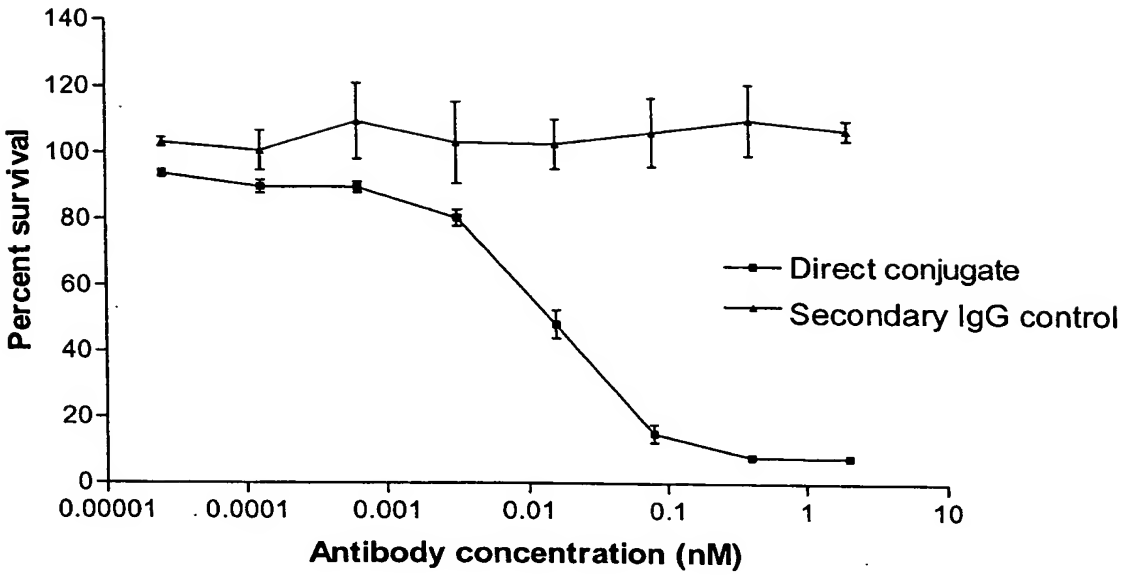

140
120
100
80
60
40
20
0
Percent survival
0.00001
0.0001
0.001
0.01
0.1
1
10
Antibody concentration (nM)
Direct conjugate
Secondary IgG control

Fig. 3

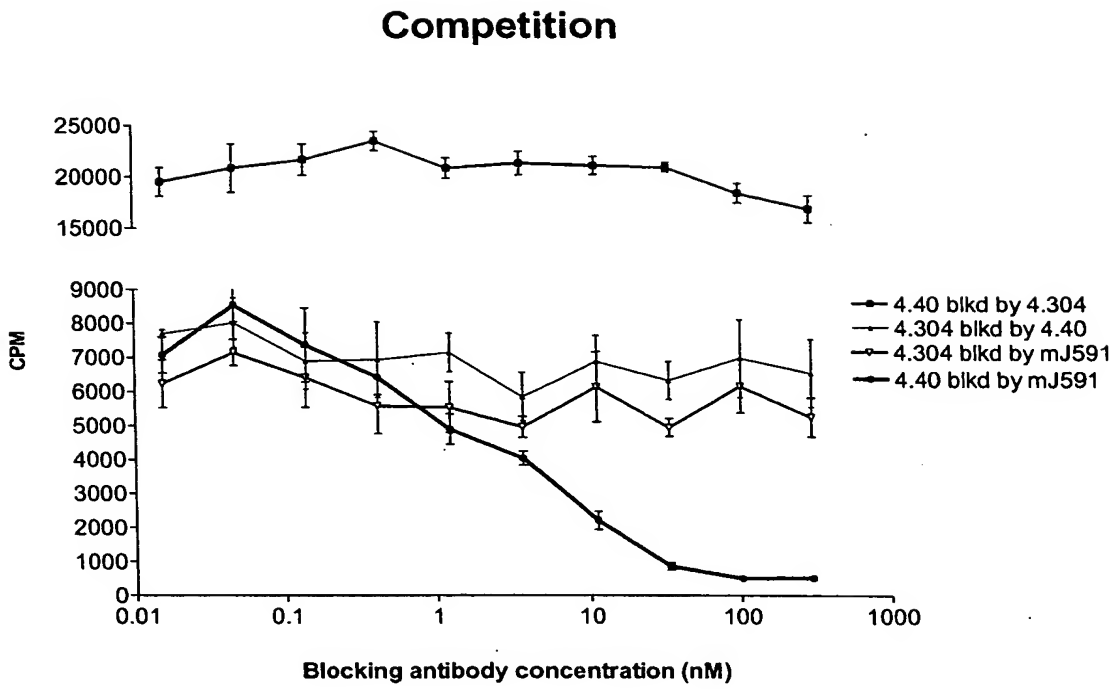

Competition
25000
20000
15000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
CPM
0.01
0.1
1
10
100
1000
Blocking antibody concentration (nM)
4.40 blkd by 4.304
4.304 blkd by 4.40
4.304 blkd by mJ591
4.40 blkd by mJ591

Fig. 32

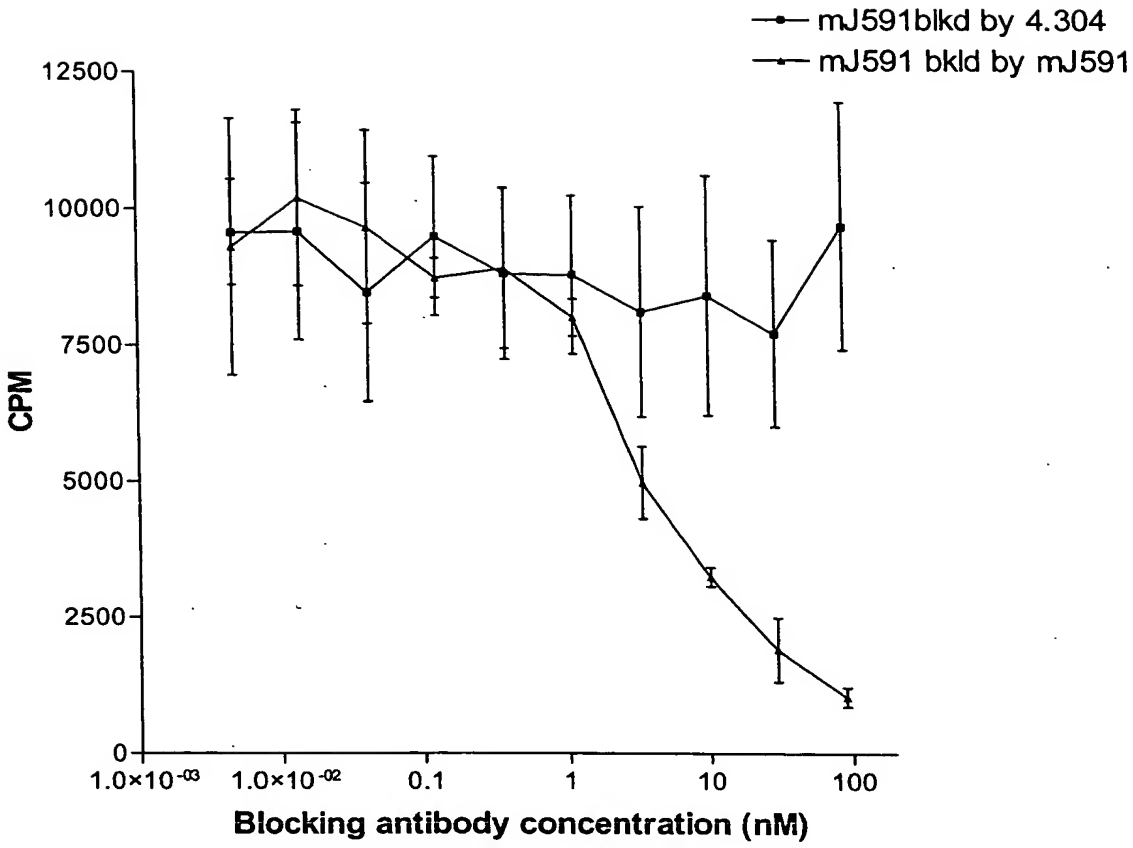

mJ591blkd by 4.304
mJ591 bkld by mJ591
12500
10000
7500
5000
2500
0
CPM
1.0×10-03
1.0×10-02
0.1
1
10
100
Blocking antibody concentration (nM)

Fig. 33

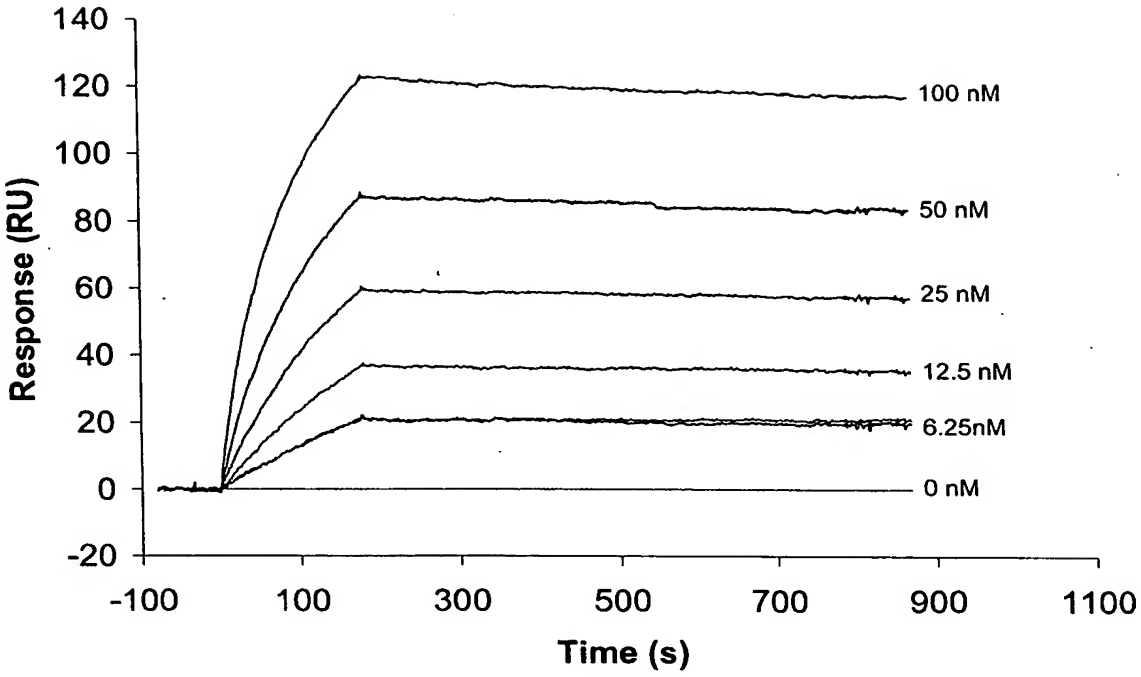

140
120
100
80
60
40
20
0
-20
Response (RU)
-100
100
300
500
700
900
1100
Time (s)
100 nM
50 nM
25 nM
12.5 nM
6.25nM
0 nM

Fig. 34

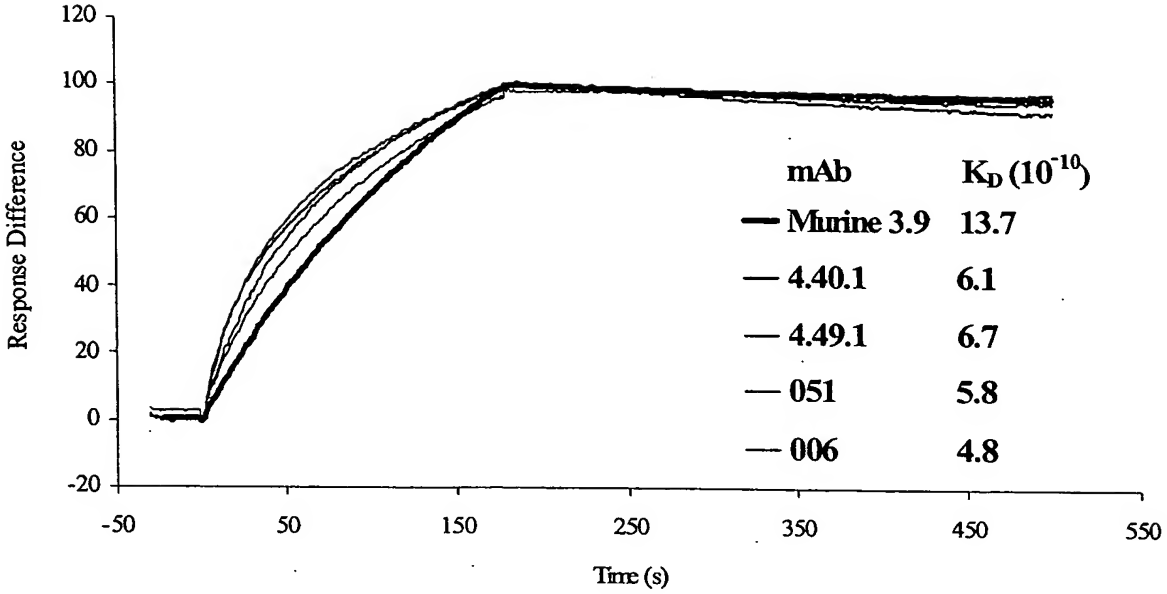

120
100
80
60
40
20
0
-20
Response Difference
-50
50
150
250
350
450
550
Time (s)
mAb
K_D (10^{-10})
Murine 3.9 13.7
4.40.1 6.1
4.49.1 6.7
051 5.8
006 4.8

Fig. 35

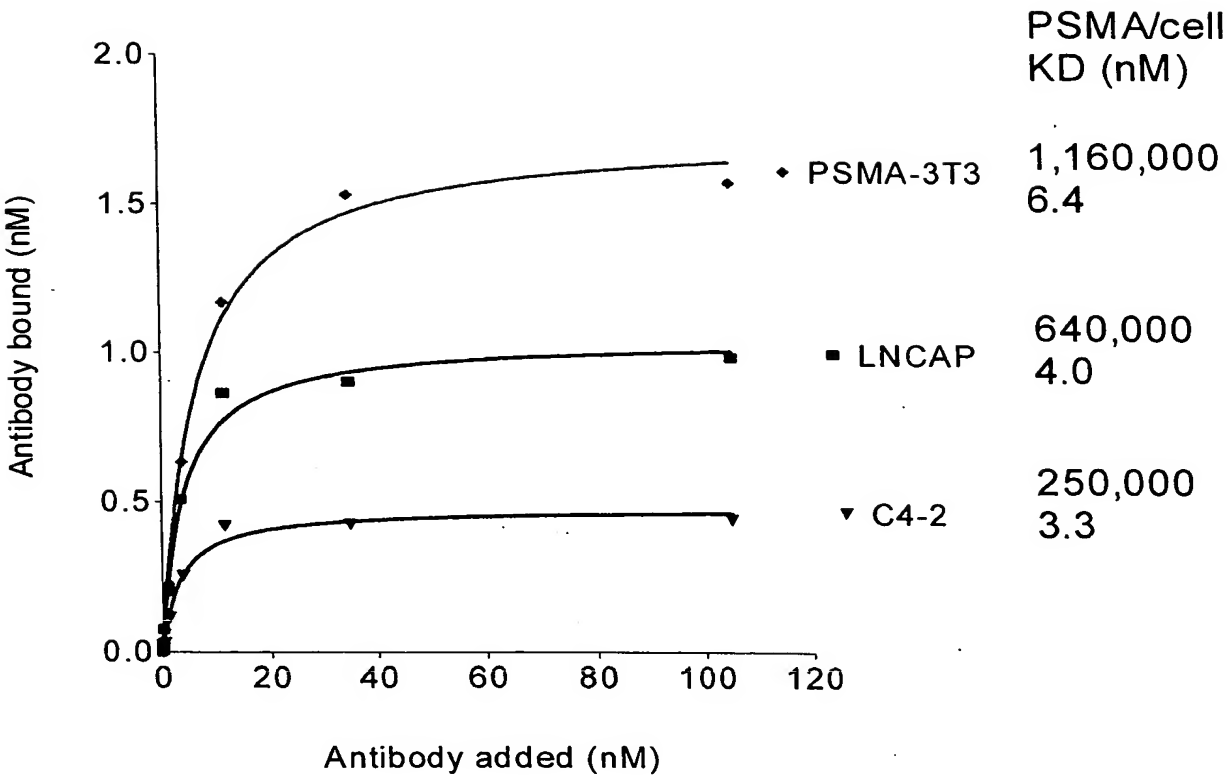

PSMA/cell
KD (nM)
2.0
1.5
1.0
0.5
0.0
Antibody bound (nM)
0
20
40
60
80
100
120
Antibody added (nM)
PSMA-3T3
1,160,000
6.4
LNCAP
640,000
4.0
C4-2
250,000
3.3

Fig. 3b

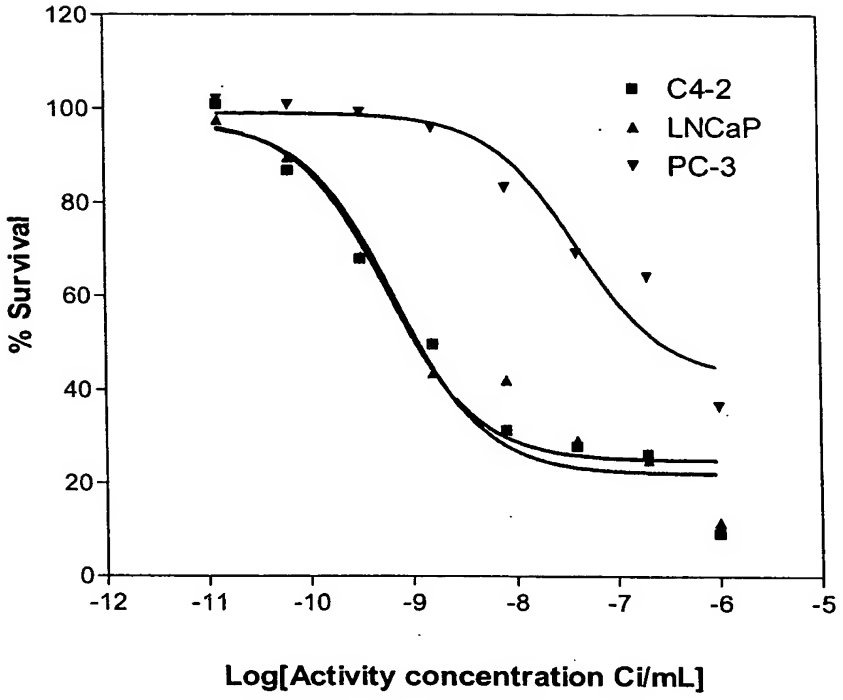

120
100
80
60
40
20
0
% Survival
C4-2
LNCaP
PC-3
-12
-11
-10
-9
-8
-7
-6
-5
Log[Activity concentration Ci/mL]

Fig. 37

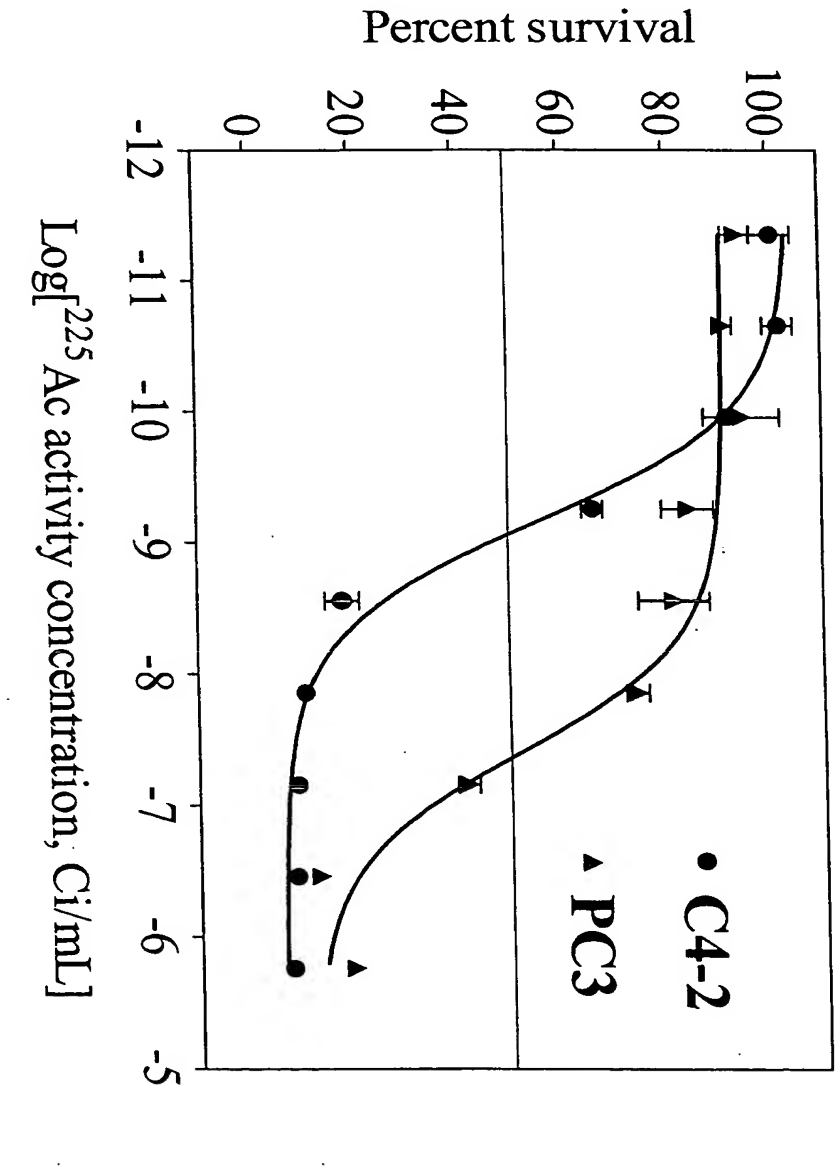
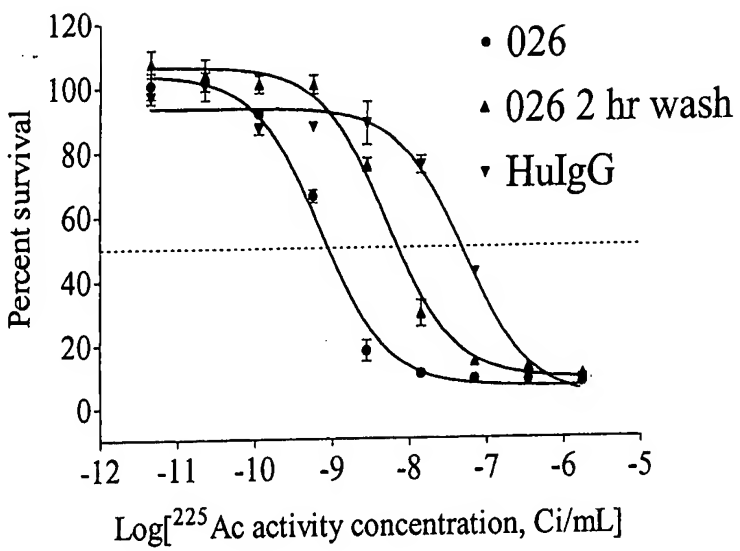

Percent survival
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
Log[^{225}Ac activity concentration, Ci/mL]
• C4-2
▲ PC3

Fig. 3B


C4-2
120
100
80
60
40
20
0
Percent survival
• 026
▲ 026 2 hr wash
▼ HuIgG
-12 -11 -10 -9 -8 -7 -6 -5
Log[225Ac activity concentration, Ci/mL]

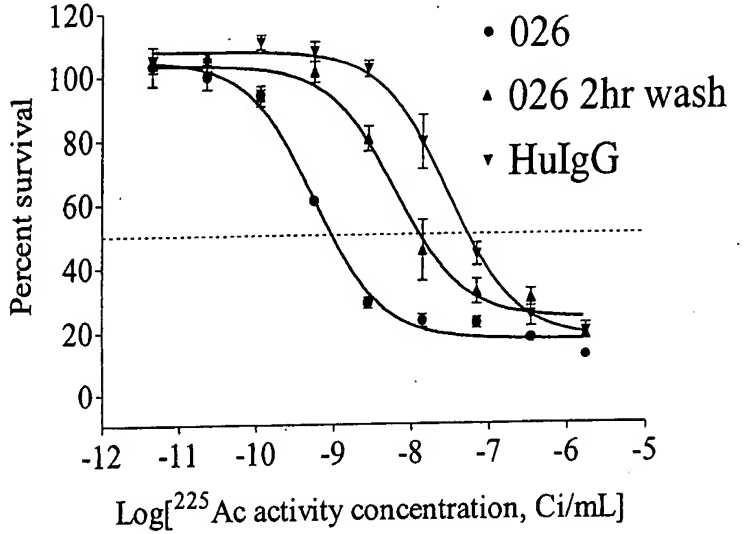

LNCaP
120
100
80
60
40
20
0
Percent survival
• 026
▲ 026 2hr wash
▼ HuIgG
-12 -11 -10 -9 -8 -7 -6 -5
Log[225Ac activity concentration, Ci/mL]

Fig. 39

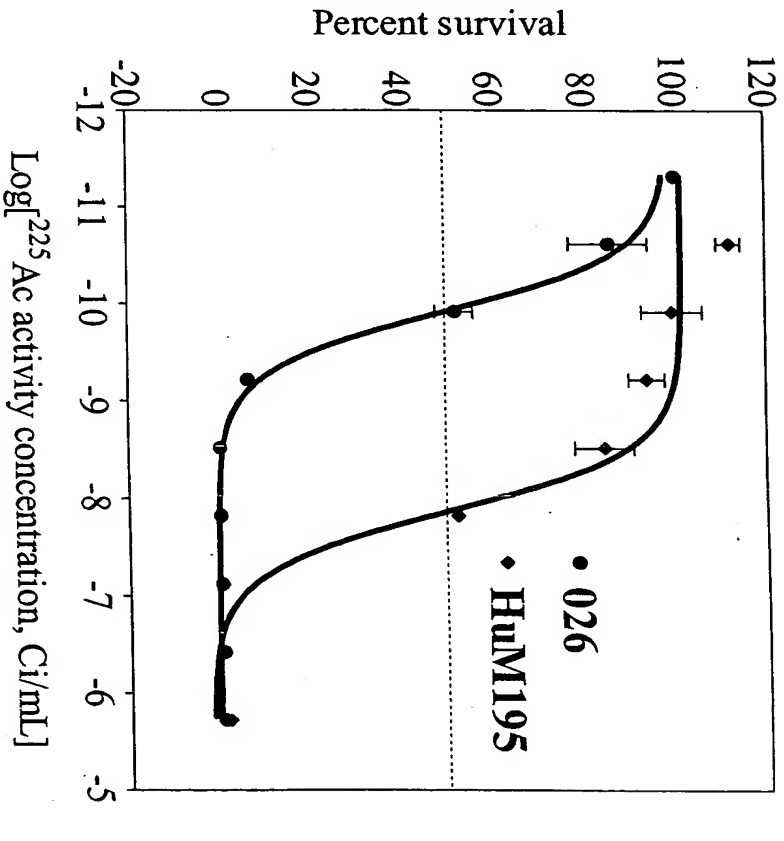

Percent survival
120
100
80
60
40
20
0
-20
-12
-11
-10
-9
-8
-7
-6
-5
Log[225Ac activity concentration, Ci/mL]
• 026
• HuM195

Fig. 40

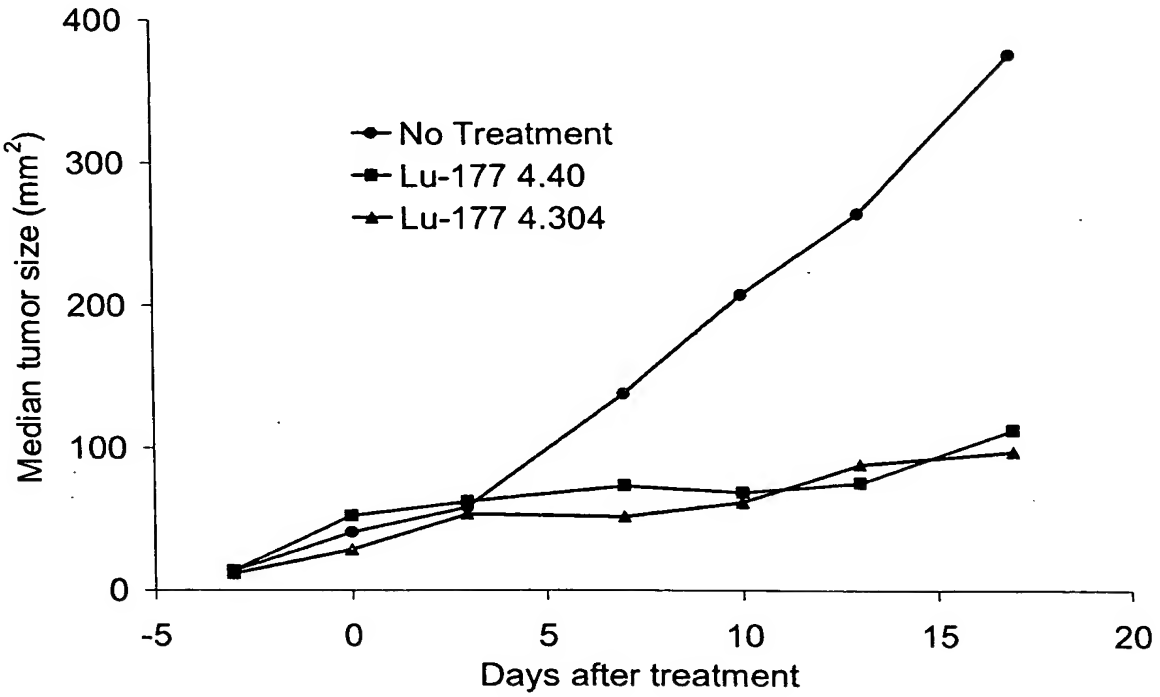
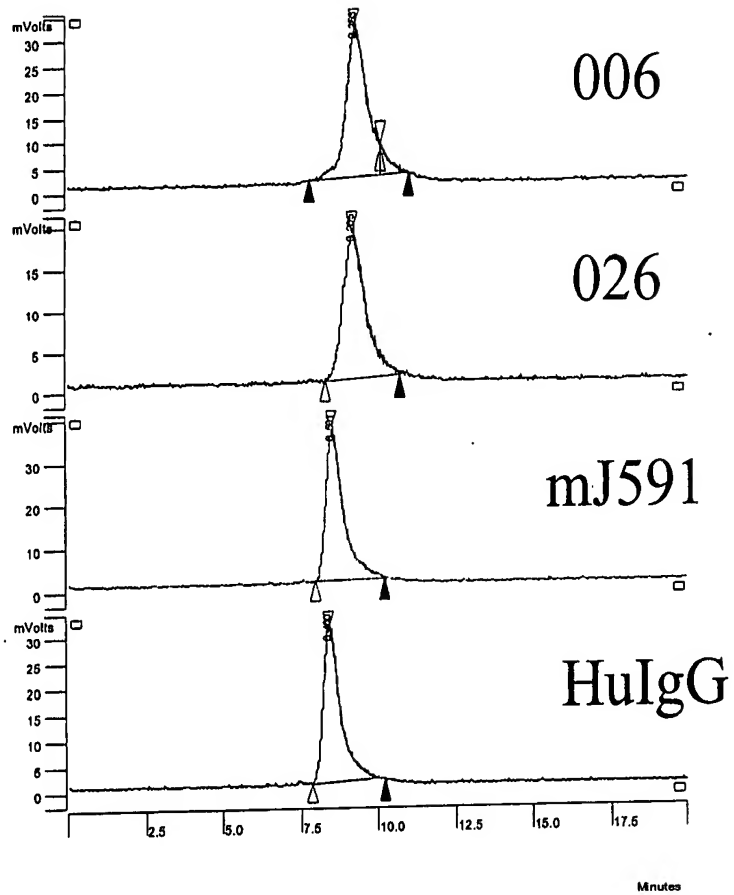

400
300
200
100
0
Median tumor size (mm2)
No Treatment
Lu-177 4.40
Lu-177 4.304
-5
0
5
10
15
20
Days after treatment

Fig. 4

Radio-HPLC profile



Cell Based Immunoreactivity (%)

mAb	PSMA	3T3
006	85.8	1.0
026	83.4	1.4
mJ591	46.2	1.7
IgG	5.9	0.7

Fig. 42

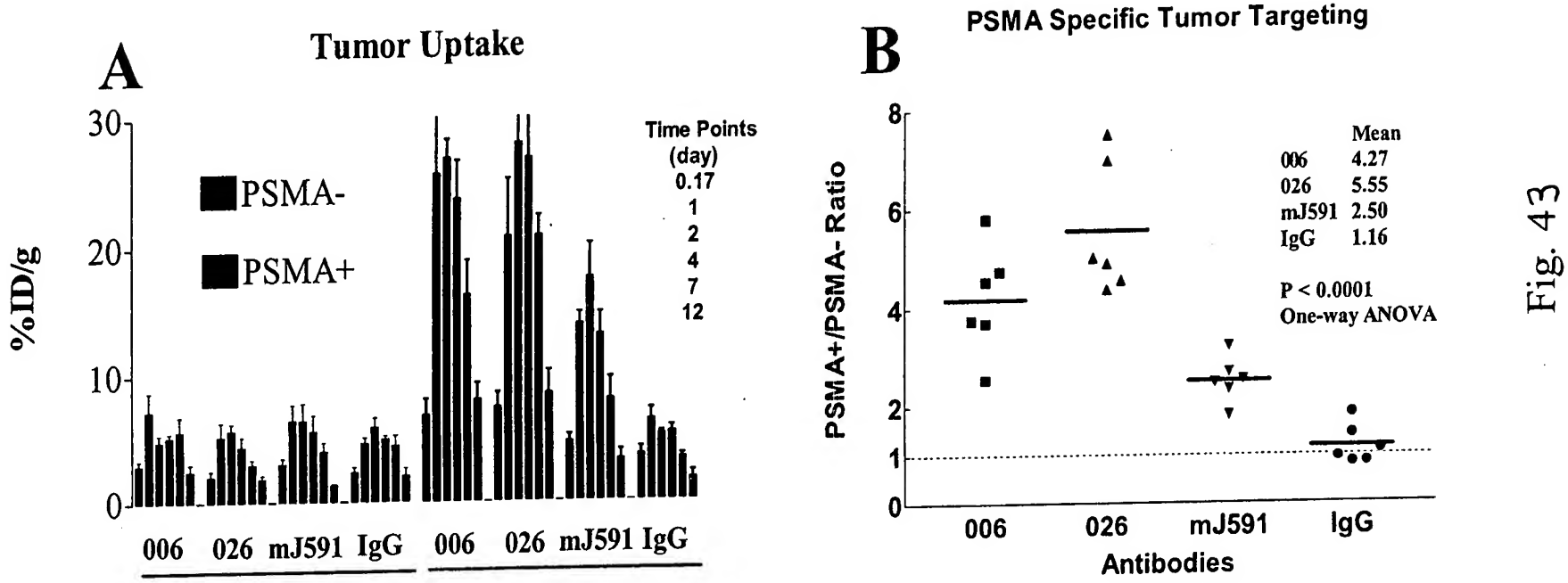
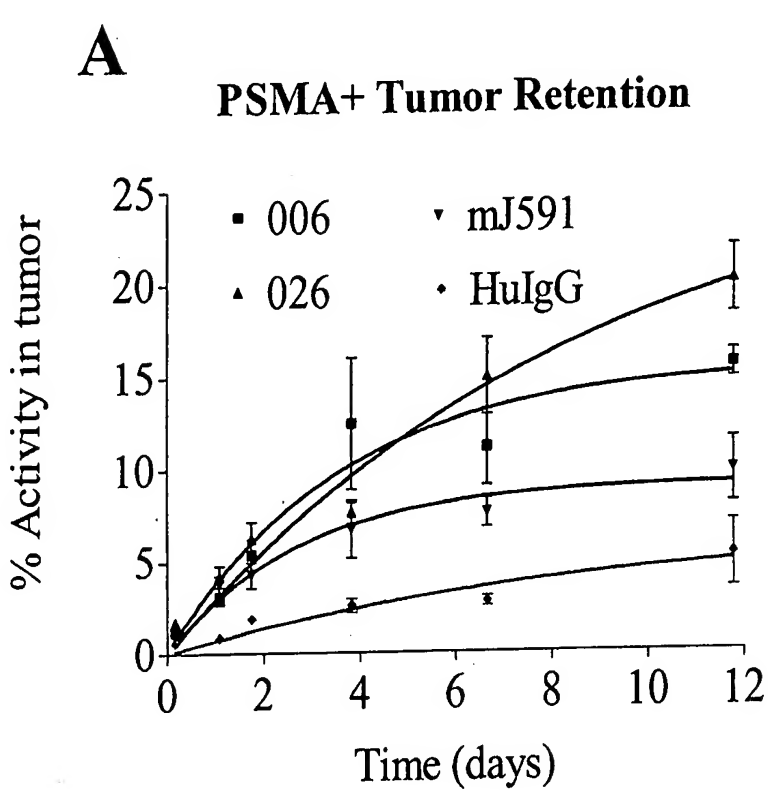

A
Tumor Uptake
%ID/g
30
20
10
0
PSMA-
PSMA+
Time Points
(day)
0.17
1
2
4
7
12
006 026 mJ591 IgG 006 026 mJ591 IgG
B
PSMA Specific Tumor Targeting
PSMA+/PSMA- Ratio
8
6
4
2
1
0
Mean
006 4.27
026 5.55
mJ591 2.50
IgG 1.16
P < 0.0001
One-way ANOVA
006 026 mJ591 IgG
Antibodies

Fig. 43


A
PSMA+ Tumor Retention
% Activity in tumor
25
20
15
10
5
0
006
026
mJ591
HuIgG
0
2
4
6
8
10
12
Time (days)

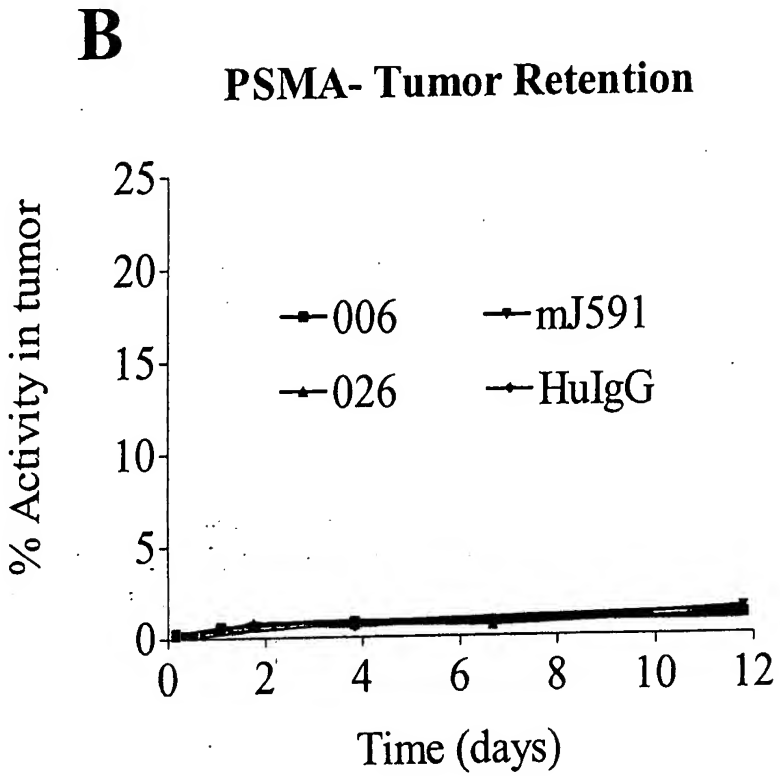

B
PSMA- Tumor Retention
% Activity in tumor
25
20
15
10
5
0
006
026
mJ591
HuIgG
0
2
4
6
8
10
12
Time (days)

Fig. 44

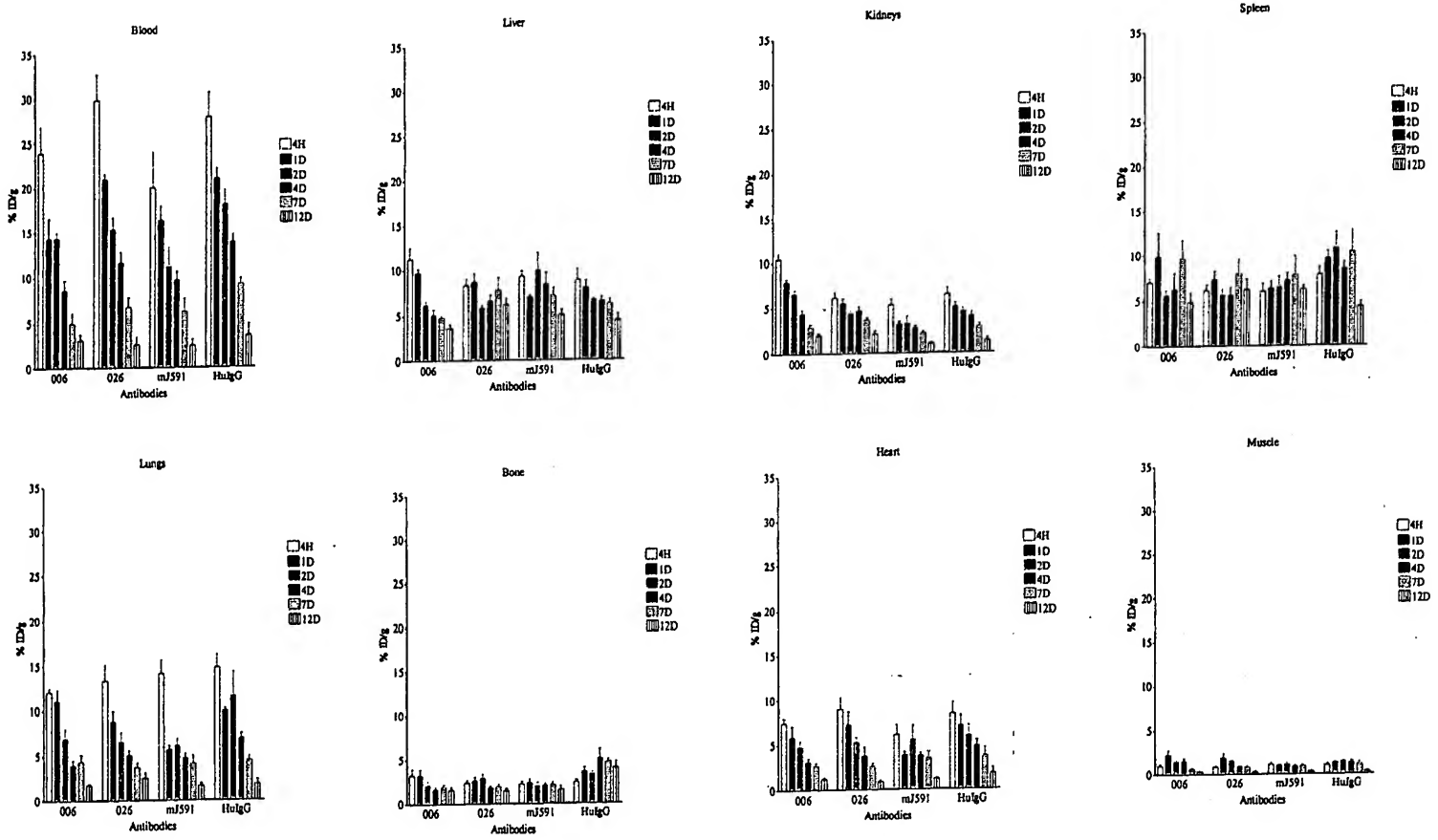
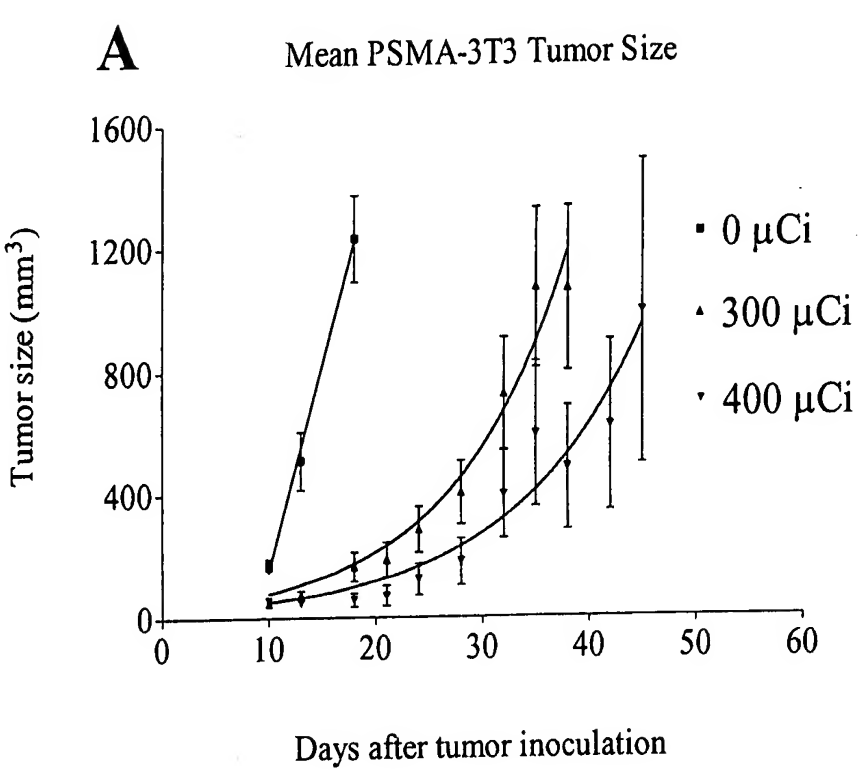

Blood
Liver
Kidneys
Spleen
Lungs
Bone
Heart
Muscle
% ID/g
Antibodies
006
026
mJ591
HuIgG
4H
1D
2D
4D
7D
12D

Fig. 45

^{177}Lu Labeled mAb 026



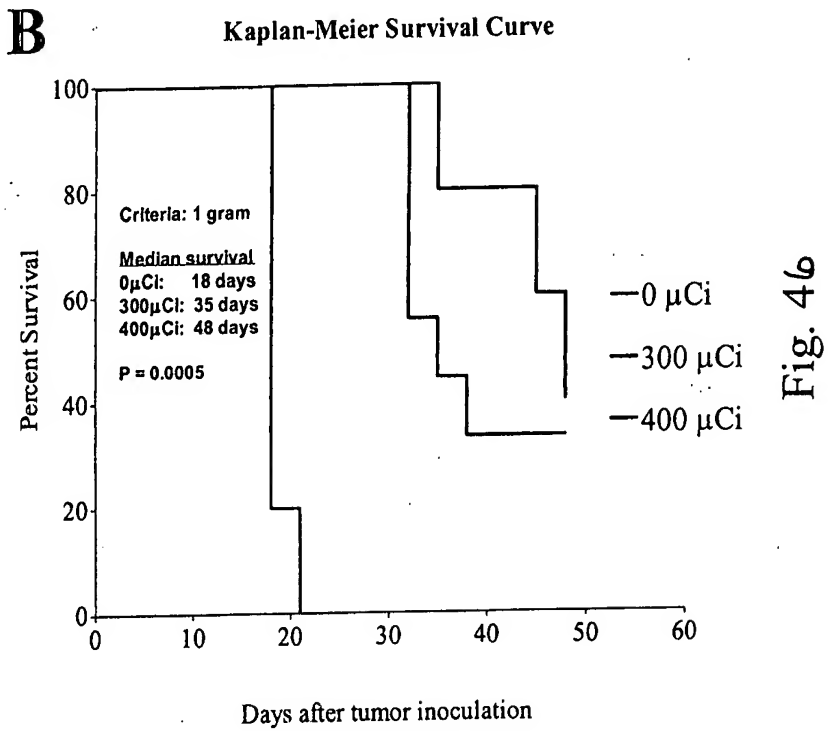


Fig. 46

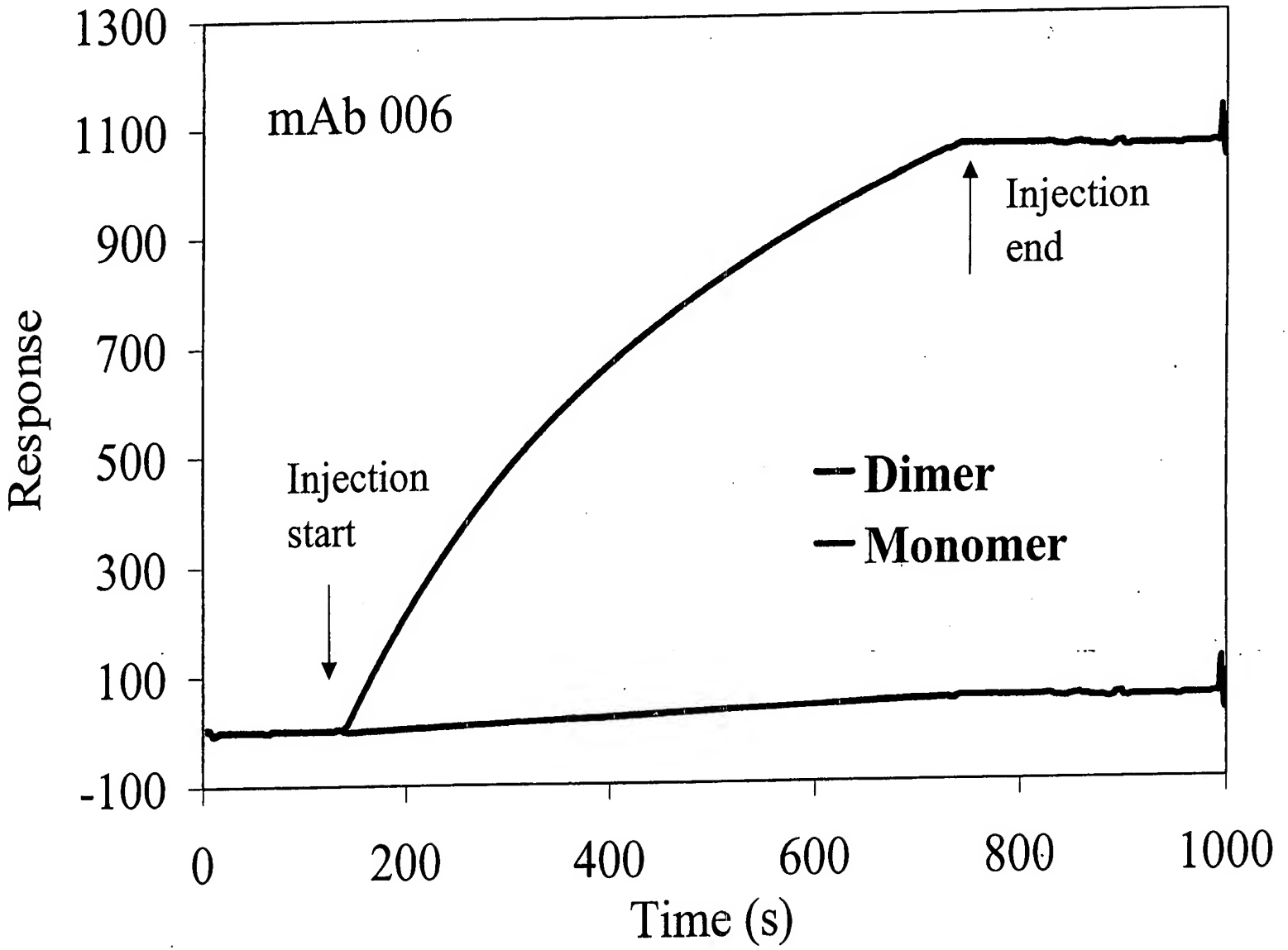

mAb 006
Injection
end
Injection
start
Dimer
Monomer
Response
1300
1100
900
700
500
300
100
-100
0
200
400
600
800
1000
Time (s)

Fig. 47

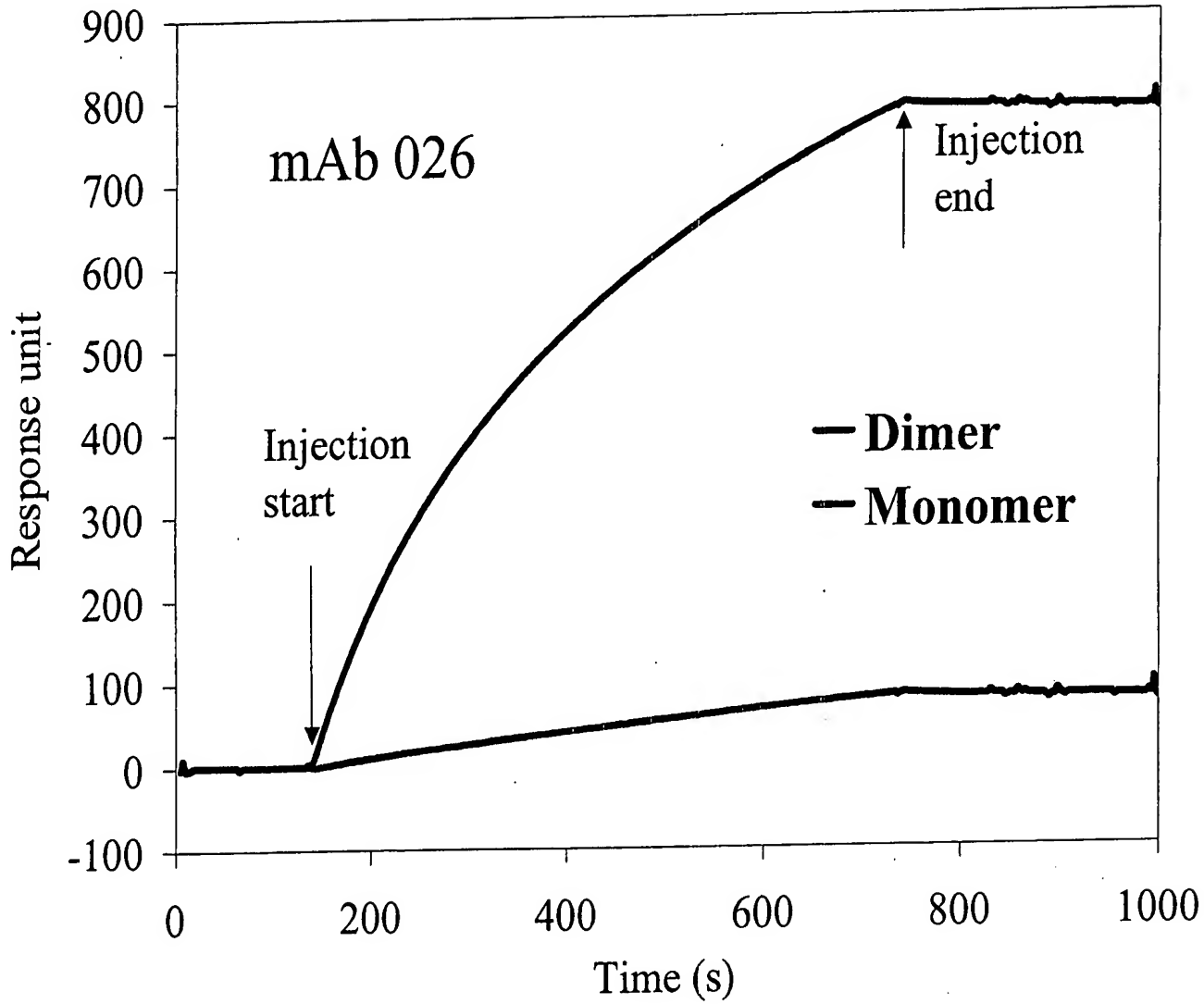

mAb 026
Injection end
Injection start
Dimer
Monomer
Response unit
Time (s)
900
800
700
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000

Fig. 4g

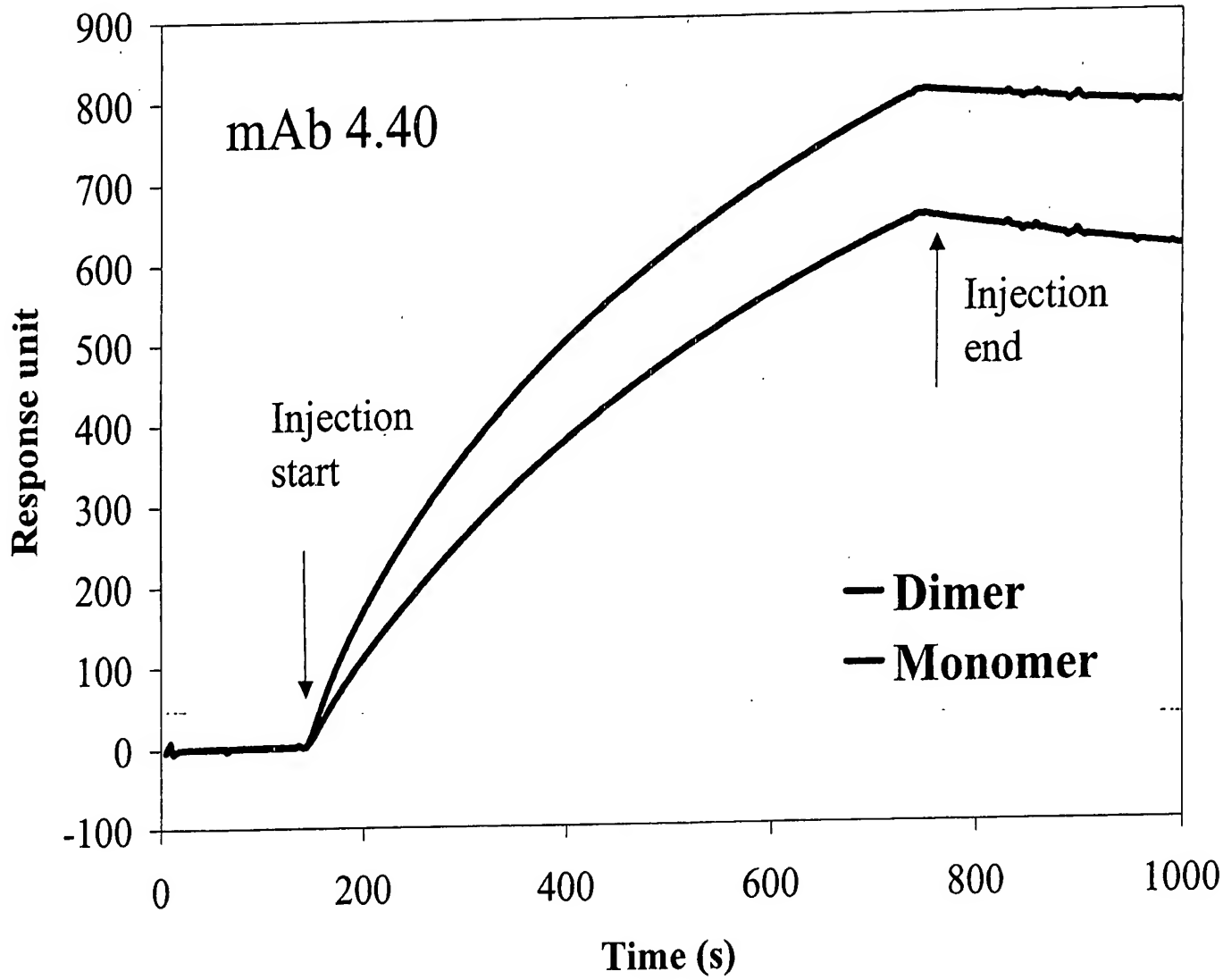

mAb 4.40
Response unit
900
800
700
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000
Time (s)
Injection start
Injection end
Dimer
Monomer

Fig. 49

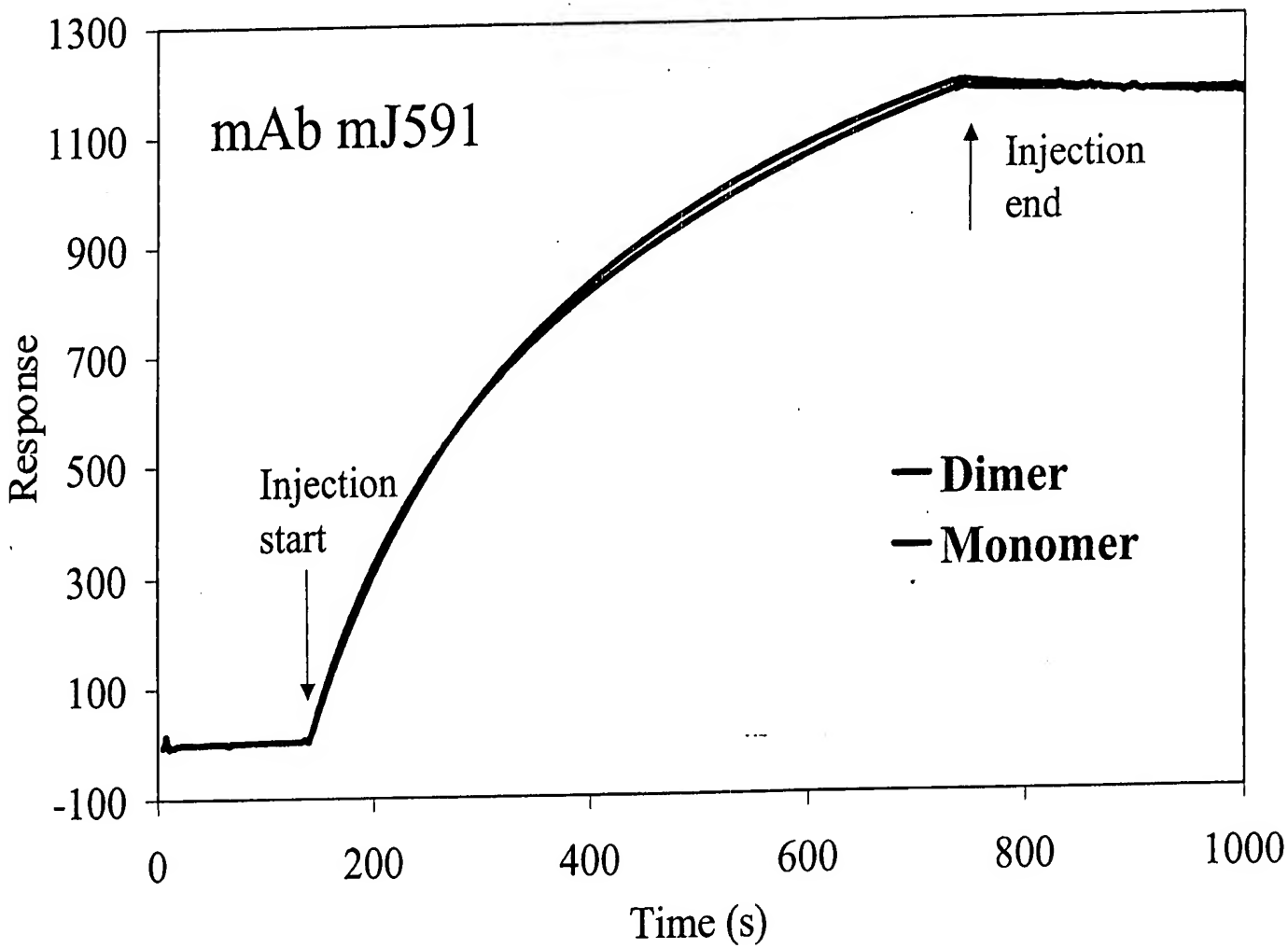

mAb mJ591
Injection end
Injection start
Dimer
Monomer
Response
Time (s)
1300
1100
900
700
500
300
100
-100
0
200
400
600
800
1000

Fig. 50

FLOW CYTOMETRY DATA

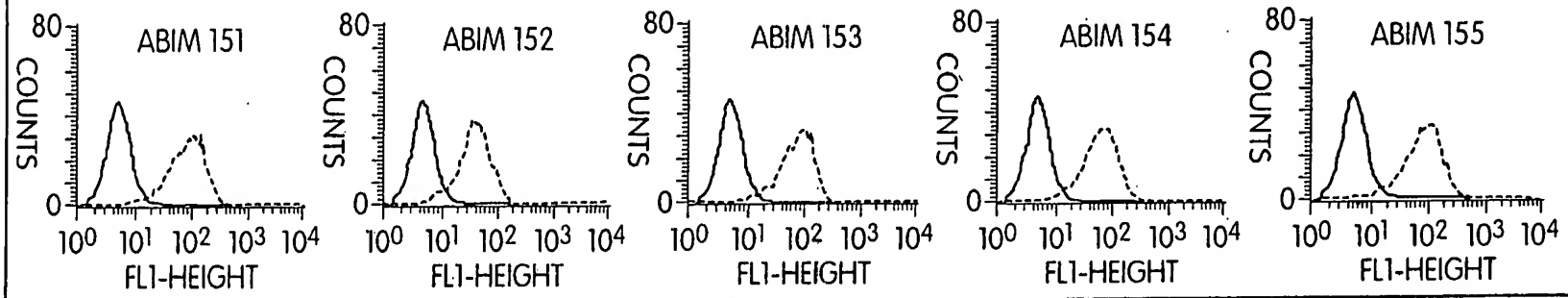
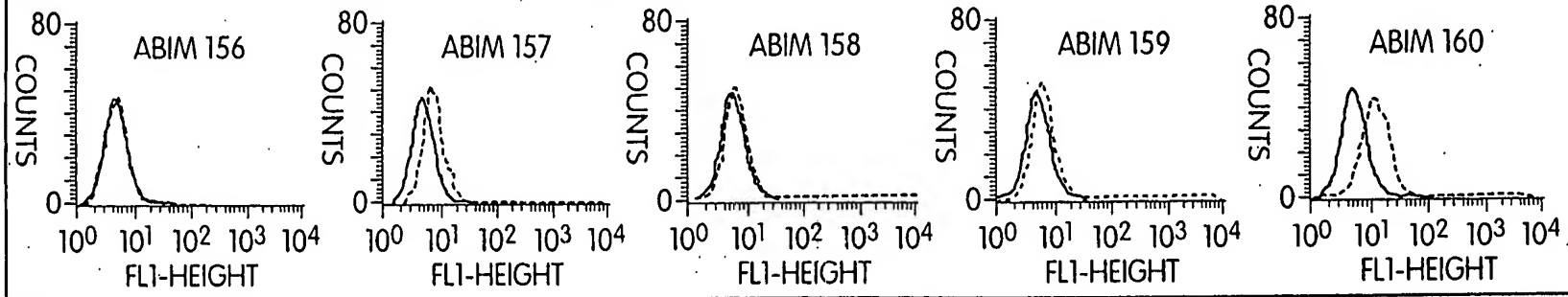
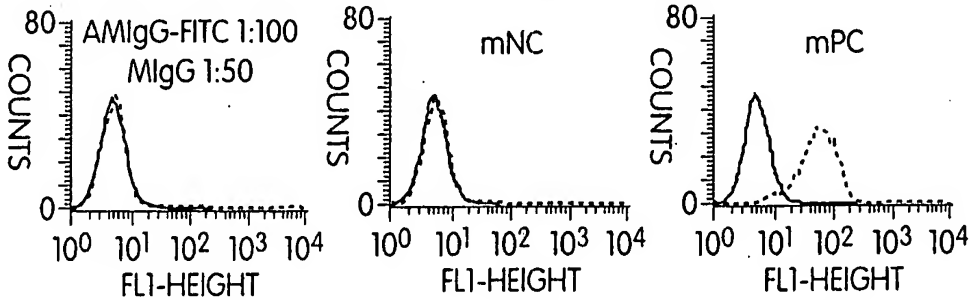
TREATMENT GROUP	RESULTS
LOT # 4019-C001	
BATCH # TD045-003 RUN1/PEAK 2	
CONTROLS	

Fig. 51

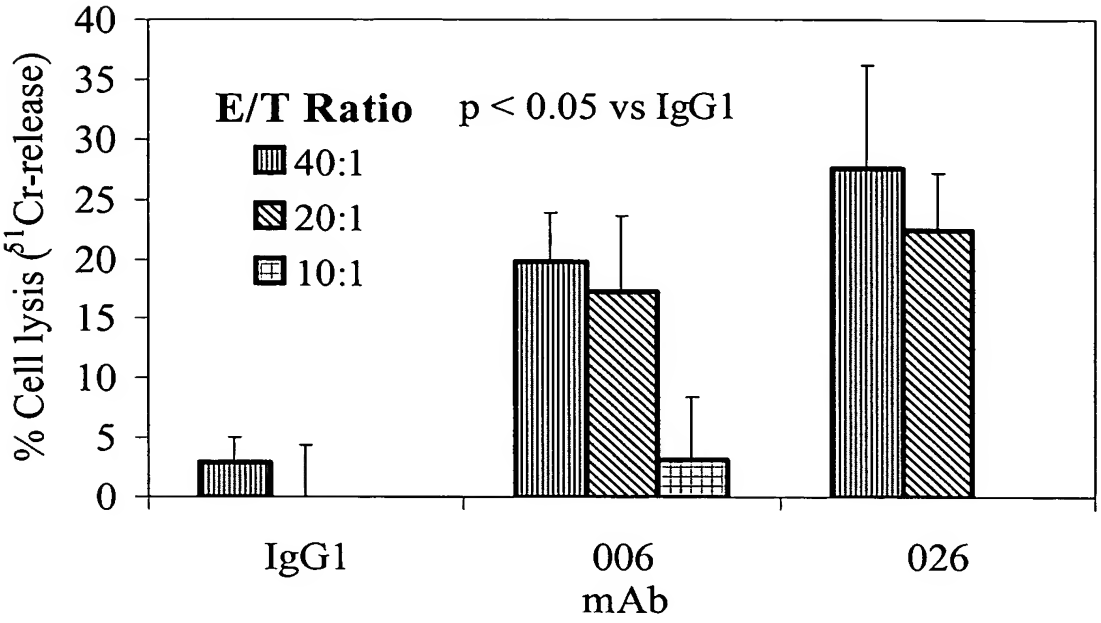

40
35
30
25
20
15
10
5
0
% Cell lysis (^{51}Cr-release)
E/T Ratio
40:1
20:1
10:1
p < 0.05 vs IgG1
IgG1
006
026
mAb

Fig. 52

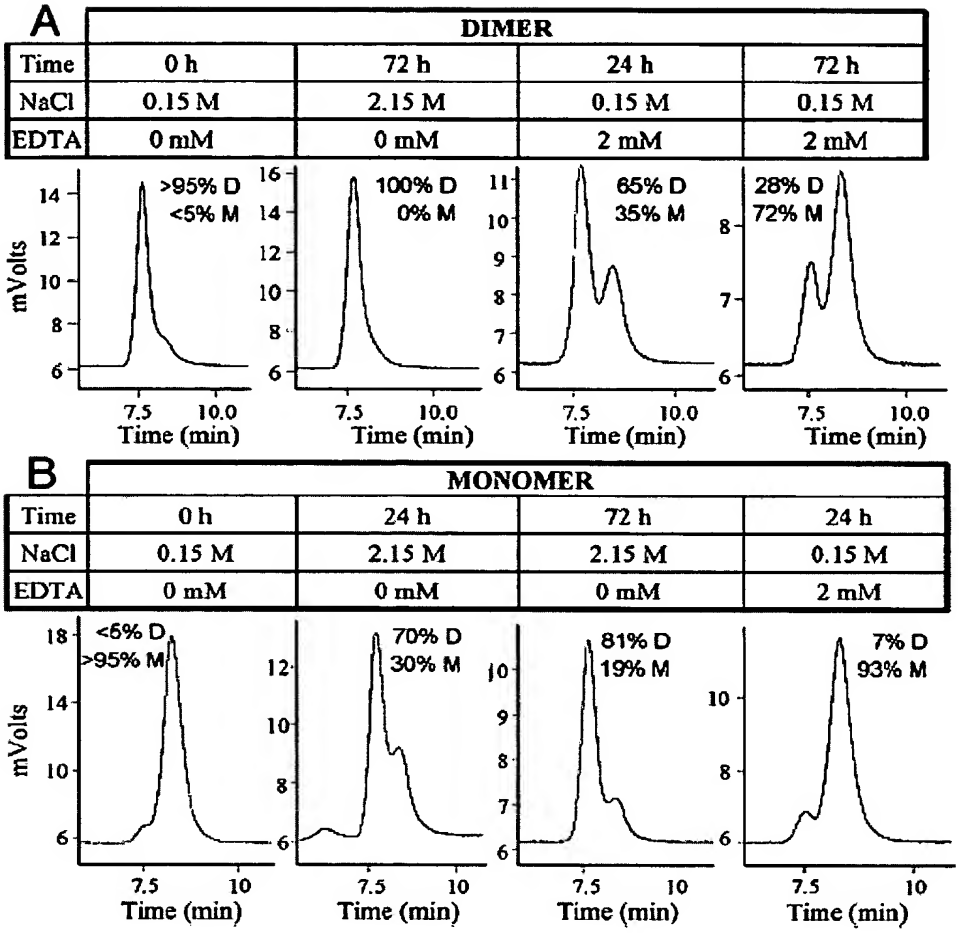

A
DIMER
Time | 0 h | 72 h | 24 h | 72 h
NaCl | 0.15 M | 2.15 M | 0.15 M | 0.15 M
EDTA | 0 mM | 0 mM | 2 mM | 2 mM
>95% D
<5% M
100% D
0% M
65% D
35% M
28% D
72% M
mVolts
Time (min)
B
MONOMER
Time | 0 h | 24 h | 72 h | 24 h
NaCl | 0.15 M | 2.15 M | 2.15 M | 0.15 M
EDTA | 0 mM | 0 mM | 0 mM | 2 mM
<5% D
>95% M
70% D
30% M
81% D
19% M
7% D
93% M
mVolts
Time (min)

Fig. 53